HOSPITAL LIBRARY

VOLUME LXXIX NUMBER 6

BULLETIN of the JOHNS HOPKINS HOSPITAL

THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL
SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY

December, 1946

A Report on an Epidemic of Mild Lymphocytic Meningitis in Burma. F. W. KIBBE, CAPTAIN, M.C., A.U.S., AND W. G. BEADENKOPF, CAPTAIN, M.C., A.U.S. 365

Studies on Schistosomiasis Japonica. 2. Analysis of 364 Cases of Acute Schistosomiasis with Report of Results of Treatment with Fuadin in 184 Cases. LT. COL. WALTER L. WINKENWERDER, MC, CAPT. ARNE V. HUNNINEN, SN. C., CAPT. THURSTON HARRISON, MC, MAJOR F. TREMAINS BILLINGS, MC, CAPT. DOUGLAS G. CARROLL, MC, AND CAPT. JOHN MAIER, MC. 406

The Problem of Identification of Oral Spirochetes and Description of a Precipitin Test for Their Serological Differentiation. LUCILLE B. ROBINSON, A.B., AND RUTH WICHELHAUSEN, M.D. 436

The Control of Experimental Pneumonia with Penicillin. II. Comparison of the Intramuscular, Respiratory and Oral Routes of Administration, with Respect to Therapeutic Activity and Rate of Absorption. CATHERINE E. WILSON, CAROLYN W. HAMMOND, 1ST LT. CWS, ANN F. BYRNE, 2ND LT. SNC, AND ELEANOR A. BLISS 451

Proceedings of the Meetings of the Johns Hopkins Medical Society 467

Book Reviews 470

Books Received for Review 479

Index to Volume LXXIX 481

PUBLISHED MONTHLY

THE JOHNS HOPKINS PRESS, Baltimore-18, Maryland

Made in United States of America

COPYRIGHT, 1946, BY THE JOHNS HOPKINS PRESS

BULLETIN
OF
THE JOHNS HOPKINS HOSPITAL

MANAGING EDITOR, HENRY N. HARKINS

ASSOCIATE MANAGING EDITOR, JOHN A. LUETSCHER, JR.

EDITORIAL BOARD

E. Cowles Andrus
Charles R. Austrian
W. Halsey Barker
James Bordley, III
John Eager Howard
Arnold R. Rich

All correspondence relating to the publication of papers should be addressed to The Editor, Bulletin of The Johns Hopkins Hospital, Baltimore-5, Md.

Books for review should be sent to The Editor at the above address.

Twenty-five reprints, without covers, of articles will be furnished to contributors free of cost. An order slip for additional reprints, with a table showing cost, is sent with proof.

Correspondence concerning business matters should be sent to The Johns Hopkins Press, Baltimore-18, Maryland, U. S. A.

This periodical is issued monthly. Two volumes a year are issued, each consisting of approximately 400 pages, and beginning in January and July, respectively.

Subscription price. In the United States and its possessions $6.00 per year. In Canada $6.25 per year; in foreign countries $6.50 per year. Prices for back volumes and back single numbers on application. Single numbers of the current volume, when available, $.75. Prices are net, postpaid.

New subscriptions and renewals are entered to begin with the first issue of the current volume. Should any issue of the current volume be out of print at the time the subscription order is received, the pro rata value of such numbers will be credited to the next volume, and the renewal invoice therefor adjusted accordingly.

Subscriptions should be renewed promptly. To avoid a break in your series, subscriptions should be renewed promptly. The publisher cannot guarantee to supply back issues on belated renewals.

Subscriptions, new or renewal, may be sent to the Publishers or ordered through your agent.

Subscriptions in the British Empire except Canada may be sent to: Baillière, Tindall & Cox, 8 Henrietta St., Covent Garden, London, W.C. 2

THE JOHNS HOPKINS PRESS
BALTIMORE-18, MARYLAND, U. S. A.

Entered as second-class matter at the Baltimore, Md., Postoffice. Acceptance for mailing at special rate of postage provided for in Section 1103, act of October 3, 1917. Authorized on July 3, 1918.

A REPORT ON AN EPIDEMIC OF MILD LYMPHOCYTIC MENINGITIS IN BURMA

F. W. KIBBE, CAPTAIN, M.C., A.U.S., AND
W. G. BEADENKOPF, CAPTAIN, M.C., A.U.S.

During the month of June, 1945, opportunity was provided to observe an epidemic of a mild form of a lymphocytic meningitis occurring among United States Army personnel in Northern Burma. Twenty-three white male soldiers were hospitalized in a United States Army General Hospital.[1] They presented symptoms, signs, and spinal fluid abnormalities similar enough to warrant the suggestion that the cases comprise a disease entity. Efforts to identify an etiological agent by the usual laboratory procedures and by methods similar to those used for the virus of lymphocytic choriomeningitis proved unsuccessful.

A review of the observations made upon this series of cases is presented. The rate at which the patients were admitted to the hospital during the month of June and the degree of similarity in symptoms, signs, and laboratory findings are shown graphically together with individual protocols. Some attempts were made to lay a ground work for further epidemiological investigation.

It is probable that there were more than twenty-three cases of this disease that developed within the area serviced by this hospital. First, symptoms may have been less severe or of shorter duration and the medical officers attached to unit organizations cared for such patients on an ambulatory or semi-ambulatory regimen. That such less severe illnesses did occur, in number at least equal to the number in this series reported, is suggested by observations of medical officers in these units. Second, several patients were probably overlooked in the early part of the epidemic because of the mild degree of their illness and spinal fluid examinations were not done. Third, the hospital did not admit patients for two weeks prior to June, 1945 (16 May to 1 June), and again for a period of about six weeks after 30 June (1 July to 15 August), coinciding with the beginning and ending of the epidemic and offering strong probability of artificial limits to the epidemic as

[1] 18th General Hospital, Myitkyina, Burma.

presented. However records of a nearby military hospital failed to show any cases of a lymphocytic meningitis during this and the ensuing two months. The British hospital in Myitkyina that cares for the Burmese, Indians, and Chinese in the area reported several cases of purulent meningitis (the physician in charge estimated 3 or 4) during May and June, 1945. They had no facilities for examining spinal fluid microscopically or by culture.

Three of the patients studied are not included in this report. They presented a disease picture identical clinically to that of this series as regards their symptoms, signs and course of illness. However, their spinal fluid showed no abnormalities.

SYMPTOMS AND HOSPITALIZATION

The admissions were scattered throughout the month of June except for two periods; in a two day period (16–17 June) six patients were admitted, and during a three day period (28–30 June) six more patients were admitted. A significant number of these two groups of men were from the same organizations, several living together in the same tents. This is dealt with later in the section concerning epidemiology.

Most of the patients were admitted to the hospital with the tentative diagnosis of "Fever of Unknown Origin". The signs of meningitis were not obvious enough to persuade the admitting medical officer to label any case "meningitis". The presence of fever and the severity of headache were in most cases the chief reasons for hospitalization.

Seventeen of the patients were admitted after three days of symptoms.

The remaining six had symptoms as follows:

Patient 1 had a headache of moderate severity for five days before admission. He remained at duty until the day of hospitalization.

Patient 8 showed a six day period of symptoms before admission. The only symptom for five days was anorexia of a moderate degree; on the day before admission he developed a headache.

Patient 9 felt feverish and had generalized aching for four days before admission. On the day before admission he developed a severe headache.

Patient 18 developed a mild frontal headache and pain in the eyes five days before admission. He felt feverish occasionally during this period. Two days before admission the headache became much more severe.

Patient 20 had coryza with a non-productive cough for one week before admission. On the afternoon before hospitalization he developed a severe frontal headache with moderate eye pain.

Patient 21 complained of moderately severe aching pain low in his back following a truck trip five days before admission. This pain persisted and three days before admission it progressed to include aching in his arms and legs and frontal headache.

The following patients had symptoms of longer duration which were not included in the charting as they seemed unrelated to the meningeal symptoms under discussion:

Patient 15 had a cold with a nonproductive cough ten days before admission which had practically subsided on day before hospitalization when he developed a severe headache.

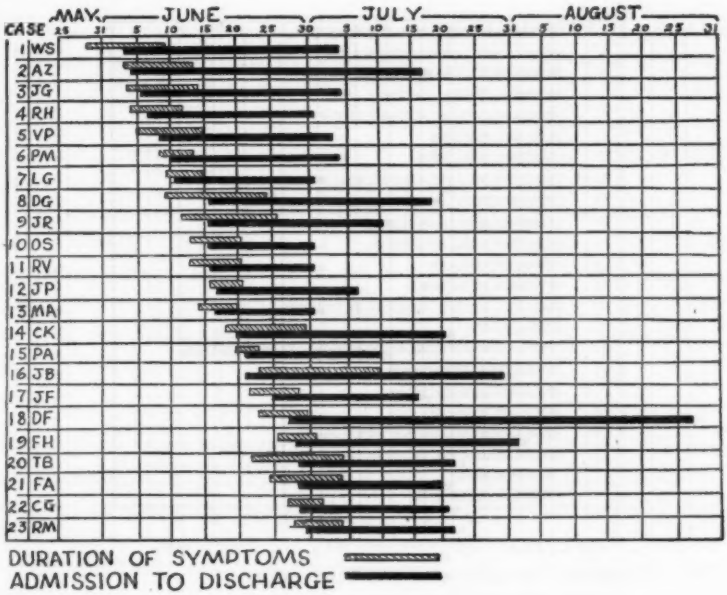


CHART 1. SYMPTOMS AND HOSPITALIZATION

Patient 16 related a history of meningitis six weeks prior to admission. He was admitted to this hospital complaining of earache and was found to have an external otitis. On the third day of hospitalization he developed a severe headache.

Patient 17 complained of slight malaise and mild anorexia for about one month prior to admission. Three days before admission he developed severe frontal headache.

The duration of symptoms after hospitalization varied a great deal, from two days in two cases up to seventeen days in one case. The average number of days of the duration of symptoms was eight and one

half. The majority fell in a five to ten day group. The charting of symptoms, both before and after admission, applies only to symptoms considered due to meningeal irritation. In two cases where symptoms were prolonged some explanation is due: 1) Patient 9 became ambulatory on the fourth day following spinal puncture, had an immediate recurrence of his headache, and was returned to bed rest until the tenth day after admission though actual symptoms did not last after

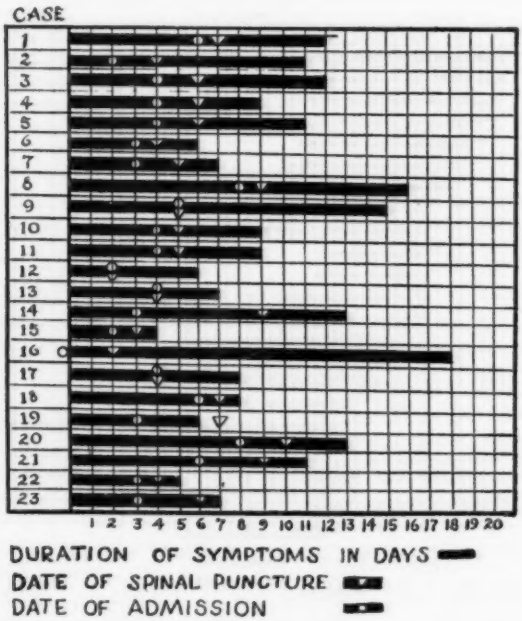


CHART 2. SPINAL PUNCTURE AND DURATION OF SYMPTOMS

the eighth day; 2) Patient 16 whose acute symptoms of headache and fever had disappeared by the sixth hospital day, continued to have pains in the ear and continued treatment for an external otitis until the seventeenth day of hospitalization.

The duration of hospitalization has little correlation with the severity of duration of the illness. An attempt was made to hold the patients about one month but this plan was altered by two conditions: 1)

the need to discharge patients because of overcrowded wards, and 2) the urgent need in some instances for the patient's presence in his parent organization.

Hospitalization was prolonged in two cases. Patient 2 was found to have amoebiasis on his seventh hospital day and treatment for it added two weeks to his hospital stay. Patient 18 was found to have amoebiasis on his twelfth hospital day. His hospitalization was prolonged also by a circumcision.

SPINAL PUNCTURE AND DURATION OF SYMPTOMS

Perceptible though not complete relief from the headache followed spinal puncture within twelve hours in 21 patients. In one patient the headache had disappeared 24 hours before a diagnostic puncture was done. In the remaining patient no improvement of the symptoms was noted until three days following removal of spinal fluid.

Three had some recurrence of headache of a moderately severe intensity about three days after the procedure. This happened when the patients were allowed up for the first time, and on return to complete bed rest headache disappeared. Three days later two of these patients resumed activities uneventfully, while the third required five days to become ambulatory.

FREQUENCY OF SYMPTOMS

The single most frequent diagnostic symptom was severe bursting frontal headache, in many instances said by the individual to be the worst pain he had ever experienced. In all cases it was present in the frontal sinus area bilaterally and behind the eyes.

Concomitantly nineteen patients stated they experienced "eye ache". Among the group of nineteen, fourteen cases noticed pain on motion of the eyes as well. An additional six patients noted photophobia.

Malaise was found commonly, manifested by generalized weakness and a tired feeling. The generalized aching and bone pain of dengue and influenza were absent although two patients had transitory backache.

Nausea was mentioned by about three fourths of the group and of these, in two thirds vomiting occurred, always to a mild degree, never necessitating intravenous replacement therapy.

Chilliness was mentioned by ten of the patients, but definite shaking chills were not described and none were seen during hospitalization.

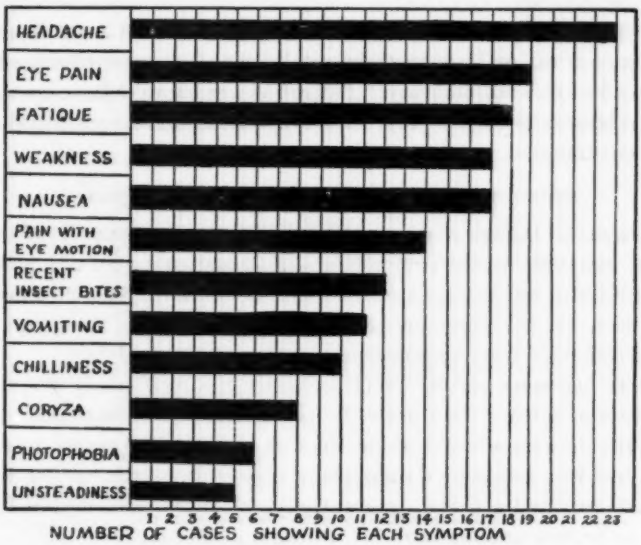


CHART 3. FREQUENCY OF SYMPTOMS

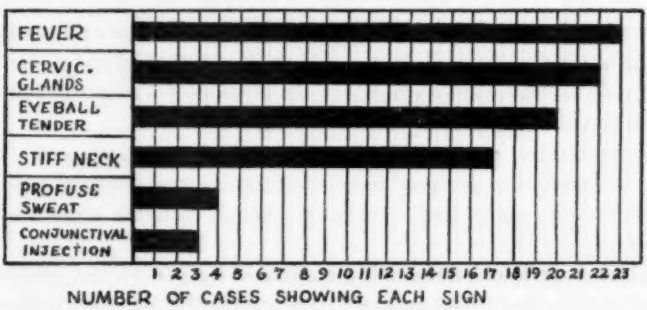

FEVER
CERVIC. GLANDS
EYEBALL TENDER
STIFF NECK
PROFUSE SWEAT
CONJUNCTIVAL INJECTION
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
NUMBER OF CASES SHOWING EACH SIGN

CHART 4. FREQUENCY OF SIGNS

What could be considered as prodromal symptoms were noted in 17 patients. Preceding coryza of a moderate degree was experienced by eight. Five had some unsteadiness on walking and slight dizziness.

The dizziness was not vertiginous in character but consisted of slight blurring of vision. Two additional patients thought that they might have had blurred vision, but were not sure because the bursting nature of the headache was so intense it minimized other symptoms. Two of the patients had diarrhea proceeding the illness but no specific organism was found during hospitalization. One of these had had diarrhea off and on for a month before admission, his last attack had occurred five days before the onset of this illness. The second patient developed diarrhea on arrival at the hospital. One other patient, who later had E. histolytica in his stool, had a mild diarrhea three weeks prior to the onset of his meningeal symptoms. The other case of amoebiasis had no diarrhea.

Twelve of the patients noticed insect bites within two weeks before admission to the hospital. Physical examination on admission failed to reveal evidence of bites or eschars of significance. These will be considered under epidemiology.

FREQUENCY OF SIGNS

The above graph depicts the occurrence of the most frequent physical signs of the described disease. In a few isolated instances other physical signs were encountered, but not in sufficient numbers to be considered graphically significant.

The cervical glands found in 22 of the cases were only slightly enlarged, pea-sized or less, and of the posterior chain. In three cases the nodes were slightly tender.

Eyeball tenderness, noted in 21 cases, was moderate, elicited by pressure directly over the pupillary area with the lids closed.

Nuchal rigidity was present in 17 cases. This finding was minimal in seven cases, mild in nine, and moderate in only one. Even in this case it was not of a degree usually found in bacterial meningitis.

Profuse sweating, even at rest, is not unusual during June in Burma. Four patients, however, appeared to perspire inordinately. There was no evidence of sweating beng restricted to either half of the body nor to any one part.

The conjunctival injection consisted of dilatation of small superficial vessels in the periphery of the sclera. It was self-limiting and required no therapeutic measures.

Mild hand tremors were found in four cases. The finding in each instance was demonstrable by holding the arms outstretched. There was no intention tremor nor any suggestion of a parkinsonian rest tremor. No other neurological abnormality was found. Reflex changes, ocular muscle changes, muscle tone variations, ataxia, hypesthesia, hypalgesia, and psychic changes were not demonstrated on any patient at any time. No convulsions were mentioned in the histories nor observed in the hospital.

Three patients had frequency of urination for two days after admission. In two cases a mild miliaria rash was noted, persistent until discharge. No other rash was seen at any time.

TEMPERATURE

The majority of the patients were admitted with fever of 100 to 103 degrees (one patient had fever of 104.2 degrees on the day of admission). The fever of each patient diminished progressively each day after admission and reached normal on the third to seventh day of hospitalization.

LABORATORY FINDINGS

White blood cell counts, hemoglobin determinations, and differential examinations showed no significant abnormalities. No abnormality of the red or white blood cells was noted. Second white blood cell counts and differential examinations were done on twelve cases. In eight, a moderate lymphocytic shift was seen from four days to three weeks after the original determinations. The greatest lymphocytic increase was from 12% to 44%. No eosinophilia of import was found.

Urine examinations were essentially negative in all but two cases which showed traces of albumin at the time of the fever. Some of the remaining patients showed an occasional white blood cell or a rare red blood cell per high power field of centrifuged sediment. All blood smears were negative for parasites, examined primarily for malaria. Blood Kahn tests, done on twelve patients, were negative.

Throat cultures were made on four indicated patients and showed the normal throat flora. No bacilli resembling diphtheria bacillus were seen on smears from Loeffler's medium. Two blood cultures were negative.

Negative cold agglutinations were found in the four patients on whom the determination was made. Proteus agglutinations done in eight cases were negative except for two: both agglutinated the OX19 strain in dilution of 1-20. (In the face of Army typhus inoculations this finding does not seem significant.)

Stool examinations in 17 cases revealed two positive for E. histolytica and one case positive for Giardia lamblia. These three were treated and subsequent examinations were negative.

During hospitalization four patients were investigated for heterophile antibody agglutinations and found to be positive in dilutions of 1:7, 1:112, 1:28 and 1:56 respectively. Because of this finding

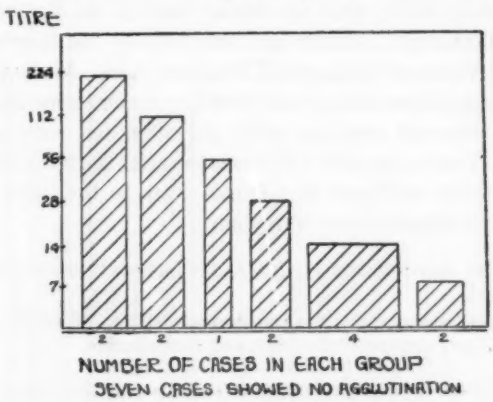


CHART 5. HETEROPHILE AGGLUTINATION

agglutinations were done on twenty of the return cases, 2 to 2½ months after the initial onset of the illness. These results are shown on Chart 5. In the case of repeat heterophile agglutinations the results are as follows:

Patient 14: 1:7 to 1:14	Patient 16: 1:28 to 1:112
Patient 15: 1:112 to 1:56	Patient 19: 1:56 to 1:7

Attempts were made to grow the infecting agent by inoculating mice. To this end ten mice[2] (white males of approximately 25 Gm.) were in-

[2] Mice were obtained from the stock of the United States Naval Typhus Commission.

jected intracerebrally with 0.03 cc.'s of spinal fluid or blood from a patient, taken within the first five days of symptoms. In all cases the blood was drawn on the same day as the spinal fluid. In six cases the spinal fluid was injected into the mouse the day it was drawn; in one case it was injected two days after withdrawal, the fluid having been refrigerated but not frozen in the interim. The blood from three patients was injected on the day it was drawn. No mice showed symptoms, either spontaneously or when stimulated with twirling by the tail. The mice were observed for a month and discarded. Two died violently (crushed by cage) on the eighth day post injection and autopsy revealed no abnormality in addition to their injuries.

Two to three weeks after the initial onset of the disease serum was taken from nineteen patients and was sent to the laboratory of the Division of Virus and Rickettsial Diseases, Army Medical Center for complement-fixation tests against lymphocytic choriomeningitis. Nine cases were reported negative while the other ten were anti-complementary.[3] Two to two and a half months after the initial illness serum was again taken and sent in a frozen state to the same laboratory. The following examinations were done:

Lymphocytic choriomeningitis: virus neutralization; 19 serum samples were all negative.

Lymphocytic choriomeningitis: complement-fixation; 18 serum samples were negative, two anti-complementary, and none positive.

Samples of serum from patients selected at random were run against the following:

Mumps: complement-fixation against two strains.

St. Louis type Encephalitis; virus neutralization.

[3] A case of a clinically identical disease was encountered one month after the reported epidemic. The laboratory findings were comparable, pleocytosis and increased spinal fluid protein. Blood serum drawn on the third day of the illness was frozen and sent to the Army Medical Center in order to isolate a virus. No virus was isolated.

The serum from a second comparable patient was sent from the 100th Station Hospital, Delhi, India, to Lt. Col. Albert B. Sabin for examination. The serum, obtained three days after the onset of the illness, was not pathogenic upon intracerebral inoculation in mice and produced no discernible illness upon inoculation in a single human subject.

Western Nile type Encephalitis; virus neutralization.
Japanese (Type B) Encephalitis; virus neutralization.
Dengue: Hawaiian strain, virus neutralization.[4]

The results of all the examinations were negative.

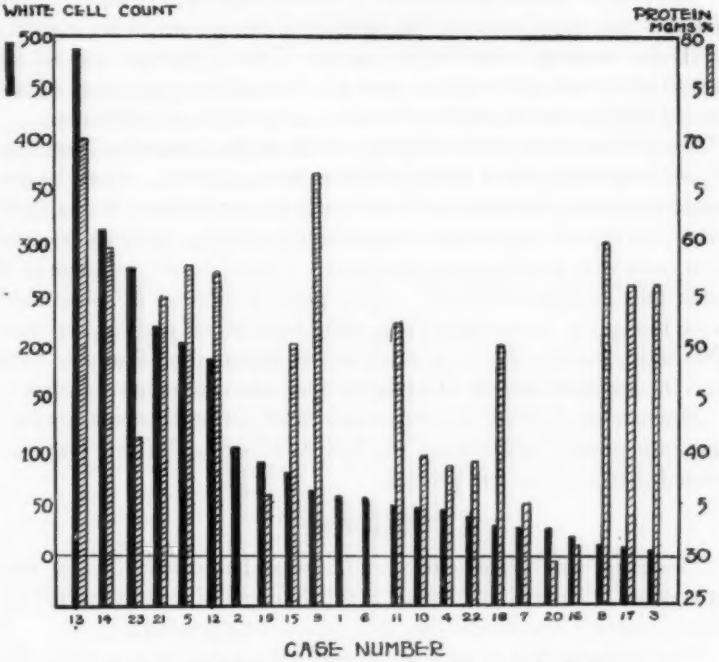


CHART 6. SPINAL FLUID CELL COUNT AND PROTEIN

SPINAL FLUID RESULTS

All patients reported showed some spinal fluid abnormality. Cell counts vary from zero to 490 cells. The highest count, 490, is the only one in which a predominate polymorphonuclear count was found, 80% as against 20% lymphocytes. The differential examination of the cells of another patient with 184 cells revealed 50% lymphocytes and 50%

[4] We are grateful to Captain R. W. Schlesinger for making these determinations in Lt. Col. Sabin's laboratory.

polymorphonuclear cells; that of seven patients showed 80% to 97% lymphocytes; that of eleven patients showed 100% lymphocytes; and the remaining three patients showed normal cell counts, viz: 3, 0, and 4 cells.

The protein concentrations varied from 29 mgs.% to 70 mgs.%. No parallel was noted between the severity of the disease or its duration with the varying spinal fluid findings. Three patients had repeat spinal punctures four weeks or more after the initial symptoms. Their spinal fluid showed normal cell counts and protein concentration.

Pandy tests were done at twenty of the original examinations; four showed one plus, six a trace, and ten were negative. Quantitative sugar analyses were done on 22 cases; in only one case was it abnormal where 30 mgs.% was found. Quantitative chloride analyses done on four cases were all within normal limits. Colloidal gold curves on fluid from four patients were 0–0. Three positive cultures of spinal fluid were thought to be contaminants, alpha streptococci and staphlococci. One smear positive for fungi could not be repeated on the same fluid and the organism seen on the original slide was considered an artifact.

Manometric pressure determinations could not be done on any cases, but with jugular compression the flow rate increased and it was presumed that no block was present.

TREATMENT

Treatment was symptomatic in all cases and no specific therapy was attempted. The self-limited nature of all phases of this disease and its short duration encouraged no therapeutic experimentation.

The headache was of such an intense nature that it frequently did not respond to codeine in doses up to 96 mgs. per dose. No morphine was given. Chloral hydrate was used for sleep as the patients seemed to rest better with it than with any of the barbiturates. Capsules of combined aspirin, phenacetin and caffeine did little to alleviate the headache in the early stages.

Lumbar puncture seemed to be of therapeutic value. In one patient, however, the headache had disappeared before the puncture was done, and in another it persisted for three days following the procedure. The remaining patients noted some relief of the headache within twenty-four hours.

One patient received sulfadiazine (22 Gm. in 5 days) for an acute diarrhea and while he was on this drug the symptoms of the meningitis became definite. It is considered unlikely that this drug has any effect on the course of the disease.

EPIDEMIOLOGY

Twenty-two of the twenty-three patients studied were questioned according to an outline made up early in the epidemic. Efforts were made to find some one factor common to all or, at least, most of them. It was not possible to make field studies in order to follow up suggestive leads presented in the questioning. Each of 22 patients (one patient had already been discharged and was not available) was questioned during convalescence along the following lines:

1. Patient's name, organization, location; how long there; previous locations for a period three months prior to illness.
2. Other nationalities in the vicinity and how close.
3. Type of work and whether day or night.
4. Type of play; athletics, swimming, animal pets, hunting.
5. Eating; whether all GI; local civilian, food from home.
6. Housing conditions; cleanliness, presence of rodents, fleas.
7. Protection against insects; wall screening, bed netting, use of insecticidal spray and repellent.
8. Friends who have been sick; who, in what way.
9. Additional remarks.

The result of the questioning is shown in the following table.

1. Living in proximity to other nationalities	22
2. Swimming in river	18
3. Ate non-GI food	13 and 1 ±[5]
4. Rodents in living quarters	13
5. Handled dogs	11 and 2 ±
6. Indoor work only	13
7. Day work only	12
8. Recent insect bites	12
9. Poor protection against insects in quarters	5 poor 6 fair.
10. Both day and night work	9

[5] The sign ± represents persons whose relation to the datum in question is difficult to evaluate in point of time; over 4 weeks and less than 8 weeks before his illness.

11. Insects in quarters	8
12. Indulged in Athletics	4 and 3 ±
13. Outdoor work only	7
14. No repellent used	5
15. On hunting trip	3
16. Both indoor and outdoor work	2
17. Handled pets other than dogs	2
18. Night work only	1

Nine patients had had contact with at least one other patient prior to their illness. Among the ground personnel of the East airstrip two patients were tentmates, while their three other tentmates did not become ill. Two other patients lived in nearby tents. These four soldiers were all admitted between the 16th and 21st of June. Two patients were tentmates who lived in Myitkyina and worked together every day. Three patients who were part of the personnel of this hospital were tentmates, while the fourth occupant of the tent complained of headache for a week but was not hospitalized. Five patients described illnesses similar to their own occurring among friends but the latter were not sick enough to be hospitalized.

Nineteen members of the series had the usual inoculations: typhus, cholera, typhoid, and smallpox, one to four months before their illness. Two had received their inoculations six months prior to admission. None had had inoculations for rabies.

The entire group of men were on atabrine suppression consisting of 0.1 gm. per day. The constancy of the intake varied somewhat with the individual.

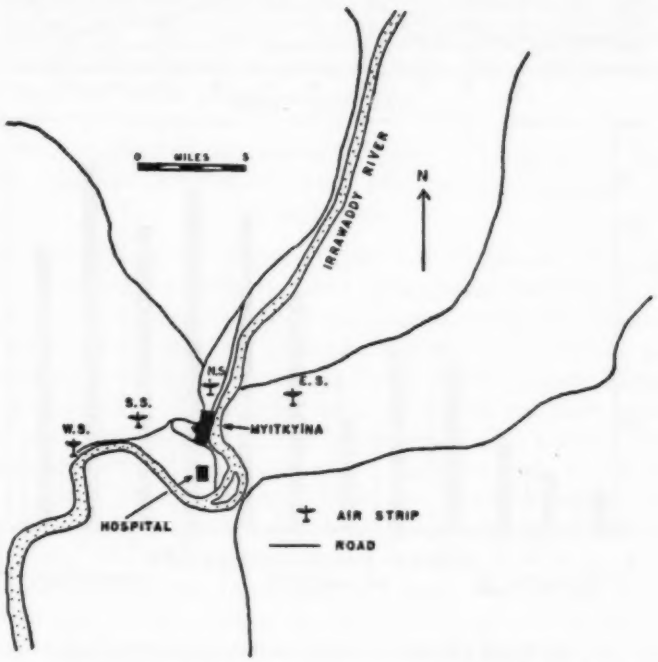
Recent questioning of six patients still available showed that none had had contact with horses, mules, or pigs. No patients came from organizations where horses were maintained. During May and June, 1945, a number of horses in the area developed trypanosomiasis; no other disease was recognized.[6] Of the entire group of patients five had contact with sick people in dispensaries and hospital wards.

The geographical distribution of the cases is shown on the map.

The twenty-two soldiers present no striking abnormalities in their location, living conditions, work or recreation from the rest of troops in

[6] We are indebted to Captain Albert F. Coates, V.C. of the 476th Quartermaster Remount Company, for supplying this information.

northern Burma, especially in this locale. Most of the population in the travelled parts of northern Burma is made up of American military, a total of about 20,000 troops in this immediate vicinity during June, 1945. About one fourth of these (estimated by hospital admissions during the month of June) are colored troops, but it will be noted that no patients of this series are colored, though the spinal fluid of two suspicious patients was examined with negative results.



None of this group was living under combat conditions. All the patients studied, like the majority of soldiers in this area, were living in semi-permanent shelters, working at a variety of occupations, all pointing toward maintaining transportation, by air and via the Ledo Road. The conditions of living are somewhat primitive, modern sanitation and sewage is impossible, and even general measures are difficult to maintain.

CLIMATIC CONDITIONS[7]

The fluctuations in temperature, rainfall and humidity for this area during the months of May, June and July are presented by weekly averages in the case of temperature and humidity, and by weekly totals

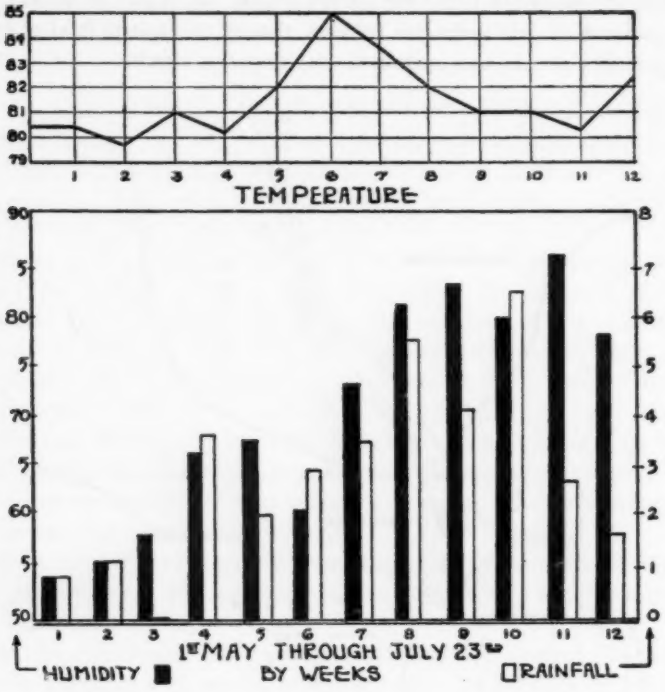


CHART 7. CLIMATIC CONDITIONS

in the case of rainfall. This has been done in order to show variants that might affect insect or other vectors of the described disease.

[7] We wish to thank Captain Richard N. Jolly, of the 10th Weather Squadron, for his extreme kindness in gathering together this detailed data from the USAAF Weather Station No. 304, South Strip, Myitkyina.

A moderately sharp rise in humidity and rainfall is noted at the start of June. Three factors may be linked with this change: 1) decrease in dust, which in the dry season is ubiquitous and pronounced, with a subsequent decrease in the fomite possibility of dissemination; 2) increase in pools and swampy land with a known increase in insects as the anopheline mosquito; 3) the tendency for snakes, rats, and mice to seek sheltered areas as living quarters. This last is unlike the habits of animals in more northerly climates where rodents are driven indoors by cold weather. This suggests a parallel to the immigration of mice indoors with the increase in incidence of lymphocytic choriomeningitis in the northern winter months.

RATE OF ADMISSION OF MENINGITIS PATIENTS IN RELATION TO THAT OF PATIENTS WITH DISEASES OR SYMPTOMS OF PROBABLE SIGNIFICANCE

The chart shows the relative number of respiratory infections, fevers of undetermined etiology, and meningitis cases admitted to the Communicable Disease Section of the hospital during the month of June, 1945. The total of the cases charted (110) represents about two thirds of the admissions (170) on the Communicable Disease Section during the month. The sixty cases not included on the chart consist of illnesses not considered significant in this report, and are made up of: malaria, dysentery, including amoebic, bacillary and unclassified forms, typhus, bronchial asthma, tuberculosis, infectious jaundice and otitis. Six cases falling into the dengue group of diseases occurred during June; four called "dengue-like" and two, sandfly fever.

The rising rate of meningitis suggests strongly that the end of the epidemic had not been reached at the end of June. In addition, it suggests that the diagnosis of the disease became more accurate and minimal cases suspected, for our cases of "fever of undetermined origin" dropped in a manner roughly proportionate to the rise in meningitis.

The significance of the increasing rate of respiratory infections is not clear. It is interesting to relate this increasing rate of infections, presumably spread by droplet contamination, to the increasing rate of relative humidity which immediately precedes the former. A breakdown of the last week recorded shows: nasopharyngitis, 13 cases, more than twice any previous week; atypical pneumonia, 7 cases,

compared with one or two cases in previous weeks; streptococcal sore throats, 2 cases; one case each of bronchitis and pleuritis.

The attempt has been made to give in some detail the setting in which these individuals contracted their disease and the relation the disease has to others occurring concomitantly. No epidemiological

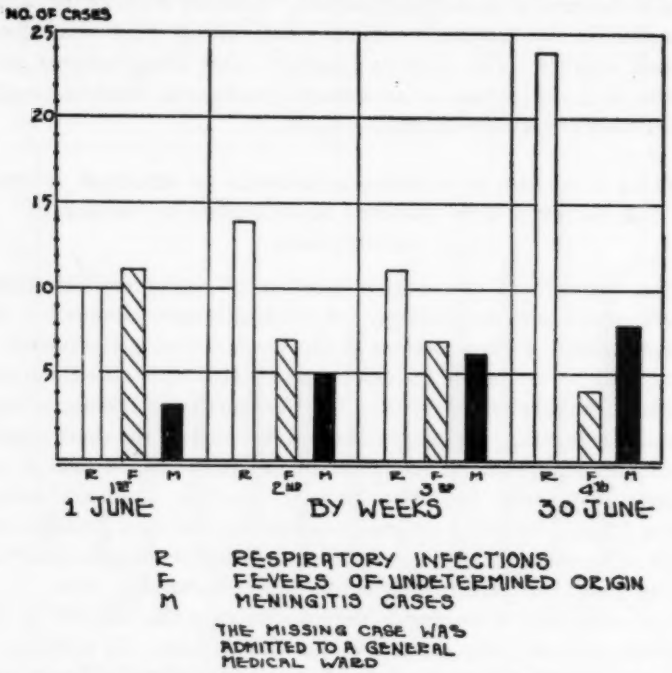


CHART 8. RATE OF ADMISSION OF MENINGITIS PATIENTS IN RELATION TO THAT OF PATIENTS WITH DISEASES OR SYMPTOMS OF PROBABLE SIGNIFICANCE

laboratory or field work could be done. The following conclusions are offered:

1. There is no clear-cut factor suggesting a mode of transmission uncovered by this study. Nor is there any significant difference between the setting and exposure of these patients studied and that of the thousands of other troops in this area. On the basis of their being

common to more than half of the patients six factors might be implicated; living in proximity to persons of other nationality, swimming in the river, eating non GI food, contact with rodents, and with dogs, and recent insect bites.

2. A moderately sharp rise in rainfall in early June resulted in a subsequent decrease in dust, a rise in humidity, and an increase in wet land.

3. The epidemic appeared among a variety of diseases no significant number of which could be related to this meningitis.

DISCUSSION

Several small epidemics of undiagnosed fevers have been described in the midst of troop movements and during seasonal changes in the past two years in North Burma and Assam. An American military general hospital in Assam[8] reports three such epidemics. Endemically from this general area we have received patients with fever of unknown etiology whose fevers vary from three to eight days duration. Many of these have some of the characteristics of dengue, with severe aching and general glandular enlargement, while others resemble "la grippe." No single distinguishing feature has been outstanding but the supposition has frequently been brought forward that they represent variants of a dengue-like disease of virus etiology. In the midst of these diseases, difficult to identify with precision, there was encountered this febrile illness with specific laboratory confirmation of meningeal inflammation. The suggestion that this illness represents a meningitis of virus etiology is based on the following considerations of a differential nature.

1. *Bacterial and Spirochaetal Meningitis.* Evidently, these are improbable.

2. *Chemical Meningitis.* Lead poisoning, as far as has been determined, is the only chemical poison, approaching epidemic proportion, that has been indicted in Burma and India. It has been found to produce signs of disease among troops working in gasoline pipeline organizations, viz: stippled red blood cells without anemia. No symptoms, neurological or otherwise, have been associated with this

[8] Colonel Francis C. Wood M.C. of the 20th General Hospital has labelled two epidemics after the patient's organizations, viz: Mule skinner's disease and 1880th Engineers' disease, while a third was called: Assam Spring Fever.

exposed group in the experience of those who have studied the problem.[9] No patients of this series were employed in pipeline organizations.

Foreign protein administrated parenterally may produce lymphocytic meningitis. This group of cases had received no immunizing inoculations for over one month prior to their illness, and the inoculations received were the usual army ones, varying in both type and lot number. Atabrine, used suppressively over long periods, has been suspected of causing or contributing toward several disorders, viz: psychoses, atypical lichen planus, metabolic alterations involving riboflavin action, liver function and pigmentation. An epedemic of a self-limited meningitis, the cases of which became asymptomatic without interrupting atabrine suppression, could hardly be associated with the use of this drug.

3. *Reactive meningitis.* A number of diseases, epidemic or endemic in nature, may produce a reactive type of meningitis; specifically: scrub typhus, mumps, measles, smallpox, psittacosis and lymphogranuloma inguinale are examples. The incidence of each of these diseases in this hospital has either been limited to five or less cases a month or none has been seen. As meningitis is a complication of only a small proportion of any of these illnesses, it would be reasonable to expect an epidemic, considerably larger in number than the number of cases of meningitis produced secondarily. Equally important is the lack of evidence for any concomitant primary disease among these patients reported. Primary atypical pneumonia has been reported as a cause of reactive meningitis (6). None of our patients had symptoms referable to the chest and in two cases where chest X-rays were taken, there was no sign of pneumonia. Amoebic dysentery has occurred in epidemic proportions in this area. Rogers and Elsom (12) reporting over 500 cases of amoebiasis in Assam describe no complication of meningitis. Bacillary dysentery did not occur in epidemic proportions and a meningitis secondary to this infection would be expected only in a small percentage of cases. The vesicles and pain of herpes zoster were not described in the history of these patients nor

[9] Captain Mitchell Miller and one of the authors (F. W. K.) found the stippling incidental to the presenting illnesses of the soldiers and later made surveys of the organizations.

were they found in the period of observation in the hospital or the ensuing two months after discharge. An epidemic meningitis, subsiding spontaneously, is difficult to associate with such disorders as: ascaris, fungi, Hodgkin's disease, leukemia, pemphigus, multiple sclerosis or Guillain-Barré syndrome. Influenza has not been diagnosed in epidemic numbers in this hospital.

While mite typhus has been seen in significant amounts in this area with encephalitis as an occasional finding, there have been no spinal fluid changes comparable to those of this disease. The other findings of typhus were obviously not found in the presented histories. No other known rickettsial diseases were seen in this area but it is possible that this represents some atypical form of a rickettsial disease of the Colorado tick fever type, though no rash was seen in any instance.

4. *Infectious Mononucleosis.* The frequency of the heterophile reaction appearing in this series suggests infectious mononucleosis. Wintrobe (15) described the disease as an acute infection characterized by irregular fever, sore throat, lymphadenopathy, splenomegaly, lymphocytosis due largely to the appearance of abnormal though chiefly mature lymphocytes, and antibodies present in the blood serum that agglutinate sheep erythrocytes. Eight patients of this series had coryza that lasted two to seven days, two of these eight had positive heterophile agglutinations with titres of 1:28 and 1:112. The other positive reactions were found in patients with no coryza. Palpable lymph glands were constant, but were limited in most cases to the posterior cervical chain. The spleen was palpated in one patient. No abnormal lymphocytes were seen in the blood smears of any patient and a relative lymphocytosis (over 40%) was found in five patients at some stage of the disease. During May and June 1945, there were two patients admitted to this hospital who were subsequently diagnosed infectious mononucleosis. Wintrobe (15) mentions Bernstein's review: "In Japan a number of epidemic fevers (tosa-netsu, tokashima-netsu, and kagami-netsu) have been linked to infectious mononucleosis by the demonstration of positive Paul-Bunnell tests." Some light is thrown on the non-specificity of this test in the work of Eaton et al. (4) on infectious hepatitis.

5. *Virus infections of central nervous system.* The disease appeared mild and of short duration compared with that usually seen in any type

of encephalitis. No patients of our series presented a history of having been disoriented, nor did any appear so under observation. There was no abnormality of the vegetative nervous system detected. There has been only sporadic poliomyelitis reported as a clinical diagnosis in India and Burma. In the absence of a clear-cut epidemic it is hazardous to report multiple cases of non-paralytic poliomyelitis.

Some agglutinations were done to rule out encephalitides of various types, but unfortunately there was no opportunity to do these for Eastern and Western equine encephalomyelitis. As Dr. Charles Janeway pointed out in a personal communication, the Eastern variety would be very unlikely, but a very mild form of the Western strain might give a somewhat comparable picture.

6. *Dengue group of fevers.* Meningitis complicating dengue fever is a rarity. Simmons (14) in his experimental work in the Philippines did not report meningitis in eighty successful human inoculations of his virus. No meningitis was described among 318 cases from the South Pacific, diagnosed clinically as dengue (7). Dengue has not been a frequent diagnosis among American troops in Burma and Assam, appearing considerably less frequently, at least in its typical form, than in the South Pacific (Fiji Islands) according to the experience of this hospital. In this present reported group, the general glandular enlargement, rash, palpable spleen, leucopenia, and saddle-back fever as found in dengue are absent.

Whenever attempting to define a mild febrile illness in this area, it is necessary to consider the dengue group of diseases (dengue, seven day fever, pappataci fever, and dengue-like fevers) with their ill-defined limits and lack of specific laboratory confirmation under ordinary circumstances. Typical dengue and seven day fever did not appear in number sufficient to supply a source for a group of cases of reactive meningitis.

Pappataci fever, as it occurs naturally (9 and 11) or induced artifically (13), has similarities to the disease under discussion: (a) the hot dry climate that is desirable for the breeding of the Phlebotomus fly, present in Burma until several weeks before this epidemic; and (b) the clinical picture of a short benign febrile illness with a bursting headache, post-orbital aching, pain on eye motion and mild nuchal rigidity. In addition, some observers (8 & 10) have associated pappataci fever

with a benign lymphocytic meningitis which took the form clinically of pappataci fever and in which spinal fluid abnormalities (increased pressure, pleocytosis and increased protein) were encountered. However, significant differences exist between pappataci fever and this illness: (a) The meningitis reported by Pearson (10) and others (9 & 11) is considered a relatively infrequent complication in the midst of a clear-cut outbreak of pappataci fever. Two patients were diagnosed pappataci fever at this hospital during the month of the epidemic, the diagnoses based upon the clinical impressions. In the extensive epidemiological survey of Sabin and his co-workers (13) the Irrawaddy River valley of Burma is not implicated as an endemic area for pappataci fever; the nearest region that has been implicated is across a range of mountains along the coast of the Bay of Bengal, a distance of over 500 miles. (b) The constant absence of lymphadenopathy in pappataci fever contrasts sharply with the constant presence of cervical adenopathy in this series of cases. (c) The absence of any constant relative or absolute decrease in neutrophilic white blood cells is significant in spite of the lack of daily blood determinations. (d) The spinal fluid findings were normal in the series of cases of Sabin et al. (13) employing known pappataci fever virus strains.

There is a probability worth investigation that some patients in the large dengue group of fevers may show atypical manifestations of infection and that this series represents an infection by a virus of a more neurotropic strain than is usually attributed to dengue or sand-fly fever, despite the fact that the sera from three of our patients showed no serological relations with the Hawaiian strain of virus. This latter is, of course, inconclusive evidence as the probability of a neurotropic strain of the dengue virus exists in view of the occasional meningeal signs and symptoms that occur in reported dengue epidemics. In some of these spinal fluid examinations were not done, while in those that were done, the findings were normal.

7. *Lymphocytic choriomeningitis.* Armstrong (1) states: "While there is evidence that strains of choriomeningitis virus from different sources may vary somewhat in their virulence for man or experimentalt animals, they have with one exception (pseudo-lymphocytic choriomeningitis) been immunologically similar." There was no immunological association of this disease and lymphocytic choriomeningitis.

Cases of both lymphocytic choriomeningitis and of this series show an acute epidemic febrile illness with signs of meningitis of varying severity, with and without neurological dysfunction and usually with a lymphocytic response in the spinal fluid.

However, there are significant differences. The influenza-like prodromal symptoms of choriomeningitis were present in but eight cases of this series, and among them the symptoms were less prominent in both duration and severity than are usually ascribed to lymphocytic choriomeningitis. Farmer and Janeway (5) quote a review of Baird and Rivers in which 18 cases of acute aseptic meningitis of unknown etiology are described as having prodromal symptoms in only three.

Lymphocytic choriomeningitis has been reported to cause neurological dysfunction often, viz: reflex changes, muscle weakness and apparent paralyses and psychic disturbances (5). None of these appeared in this series. Nuchal rigidity was less marked than that described in choriomeningitis. The symptoms of lymphocytic choriomeningitis are usually of longer duration than were observed in these cases. The pleocytosis of choriomeningitis (600 to 3000 cells) is higher than was encountered among these patients. The virus of choriomeningitis is highly fatal to non-immune mice. Here our results are of doubtful negative value. Though our mice showed no reaction to injection of infected blood or spinal fluid, we have no evidence to show that the mice were not immune. Complement-fixation and neutralization tests were negative for lymphocytic choriomeningitis. Lymphocytic choriomeningitis is reported to occur in the autumn, winter and spring. This epidemic reported, though present in June, may not have been limited to that month. Cellular reactions in the spinal fluid of choriomeningitis are reported to persist four to six weeks and as long as four to six months. There was no abnormality of the spinal fluid found in three patients of this series on repeated spinal fluid examination one to two and one half months following the onset of their illness. In general, the reported disease is a milder shorter illness than lymphocytic choriomeningitis and has shown no neurological dysfunction at any stage, a frequent part of lymphocytic choriomeningitis.

9. *Other aseptic meningitides of known etiology.* The literature available does not permit an evaluation of the similarities and differences clinically beteeen our series and the other lymphocytic meningitides of known etiology. In the case of Bouchet's disease, "la maladie des

porchers," there is little resemblance. As described by Farmer and Janeway (5) there is the exposure to pig excreta and the typhoid-like pre-meningeal phase with a rash, neither of which were found in our patients. The disease produced by the virus of MacCallum, Findlay, and Scott (pseudo-lymphocytic choriomeningitis) is not described in detail, nor is there information available on the appearance of the diseases resulting from other viruses as : virus D of Durand and the virus of Enders and Liu (5).

Considering the clinical and laboratory data described it seems most appropriate to include this series in the group of benign meningitides described by Wallgren in 1925, and named by him "acute aseptic meningitis." The many cases reported since 1925, though frequently suspiciously of virus origin, have definitely been associated with specific viruses in about one third of the total number (2). The cases of this series fall into the larger division of the group, those of unknown etiology.

There remains a probability, in our opinion slight, that the meningitis reported here might be a reactive meningitis secondary to some systemic virus infection, due to the presence of the organism itself or a toxic product of the organism within the central nervous system. In this event, the type of virus would most likely fall into the dengue group of viruses. The isolation of such a causative organism, or its identification serologically, is necessary to settle the point and through these means the disease could be definitely classified.

SUMMARY

1. A report is presented on a group of 23 patients with a mild form of lymphocytic meningitis, without residua, that occurred in northern Burma during June, 1945.

2. The principal symptom was severe frontal headache; the chief signs; fever, posterior cervical lymphadenopathy and eyeball tenderness. Laboratory examinations showed spinal fluid abnormalities of pleocytosis or increased protein or both.

3. Epidemiological studies are presented; no definite conclusions could be drawn.

We wish to express our appreciation to Lt. Col. Herman L. Blumgart, M.C., Major Edgar F. Cosgrove, M.C., and Lt. Col. Fred R.

Sloan, M.C. for their invaluable aid and cooperation, and to Lt. Col. Joseph E. Smadel, M.C. and Lt. Col. Albert B. Sabin, M.C. for their kindness in performing special laboratory determinations.

The technical assistance of Pfc. Louis F. Dydo and the draftsmanship of T/4 Willard D. Clark in making the charts have added considerably to the presentation of the data in this paper. We wish to thank them.

Case no. 1 (W. S.): Hosp. Reg. No. 14571. A 20 year old white male, admitted 3 June complaining of severe headache.

P.H.: Non-contributory.

P.I.: This began 5 days before admission with a headache which became increasingly severe. One day before admission he developed a sore throat and fever.

P.E.: A mild erythema of the chest was noted, said to be due to sun exposure. There was slight injection of the throat and a few small cervical glands palpable bilaterally in the posterior triangle of the neck. There was some questionable neck rigidity. No eyeball tenderness was found.

Course: A lumbar puncture was done the day after admission with some relief of the headache. Temperature went to 101.2 and continued somewhat elevated for 2 days after hospitalization, and then became normal. Headache, diminished after the puncture, continued for 6 days after admission and then cleared. After the patient had completely recovered from his illness he received ultra-violet therapy for a mild acne that was present over the shoulders, upper chest and face.

Laboratory findings: Hgb., 90%. 3 June: WBC, 11,700; seg., 61%; lymph., 27%; eosine, 2%. 7 June: WBC, 10,300; seg., 47%; lymph., 47%; eosin, 2%. Urine negative. Spinal fluid: 50 lymphocytes, Pandy negative, Sugar 84 mgs. %. Culture and smear for organisms negative. Throat culture: normal throat flora on Loeffler's no K-L organisms seen. Blood smear: no malaria parasites. Blood Kahn: negative. Serum was drawn for complement-fixation test against lymphocytic choriomeningitis on the 28th day of hospitalization; reported anti-complementary. Serum was also examined for neutralizing antibodies against the Hawaiian strain of Dengue, and no antibodies were found. The patient was discharged 4 July having shown no residua of the infection.

Examination 3 months after the illness revealed no recurrence or residua. Serum was drawn at this time and tests performed. The tests and their results follow: Lymphocytic choriomeningitis: complement-fixation and virus neutralization, both negative.

Case no. 2 (A. Z.): Hosp. Reg. No. 14608. A 21 year old white male, admitted 4 June complaining of headache.

P.H.: Non-contributory.

P.I.: The illness began with frontal headache the night before admission, and became more severe during the night.

P.E.: There was slight injection of the pharynx; small tender posterior cervical nodes and a minimal nuchal rigidity were present. The spleen was palpable about one finger-breadth below the costal margin. No eyeball tenderness was noted.

Course: A lumbar puncture was performed on the third hospital day. The following day the headache was considerably less severe. Mild diarrhea developed in the second week of his hospitalization and the stool was found to be positive for E. histolytica. This was treated with routine therapy. The patient was symptom free by the 10th hospital day.

Laboratory findings: Hgb., 85%. 4 June: WBC, 8,300; seg., 88%; lymph., 12%. 7 June: WBC, 5,100; seg., 56%; stab, 3%; lymph., 34%; mono., 4%, eosin., 3%. Urine negative. Spinal fluid: WBC 103, all identifiable were lymphocytes, Pandy negative, sugar 63 mgs. %, smear and culture showed no organisms. Blood smear: no malaria parasites. Proteus agglutination: negative. Stool examination: E. histolytica; none found after treatment. Serum was taken for complement-fixation against lymphocytic choriomeningitis on the 25th hospital day; reported negative.

The patient was discharged well in 17 July. Follow-up examination done 2½ months after the initial onset showed no residua. Heterophile agglutination at this time was negative. Serum was drawn at this time and tests performed. The tests and their results follow: Lymphocytic choriomeningitis: virus neutralization negative. Mumps: complement-fixation negative.

Case no. 3 (J. G.): Hosp. Reg. No. 14716. A 23 year old white male, admitted 6 June complaining of headache.

P.H.: Non-contributory.

P.I.: A generalized headache developed three days before admission. Anorexia developed two days before admission accompanied by a slight fever. The headache became localized over the eyes, followed by nausea and vomiting. In addition, he had a slight soreness of the throat.

P.E.: Pain on pressure and motion of the eyes was noted. No nuchal rigidity nor cervical adenopathy was found.

Course: A lumbar puncture was done on the third hospital day, following which the headache decreased in intensity and the vomiting, which had been present until that time, ceased. The fever which at its height was 100 dropped to and stayed at normal from the third hospital day. His symptoms cleared entirely by the eighth day after admission.

Laboratory findings: Hgb., 90%. 7 June. WBC, 6,150; seg., 70%; lym., 27%; stb., 3%. Urine: negative except for an occasional white cell. Spinal fluid. WBC 0, RBC 47, Pandy trace, Protein 56 mgs. %, Sugar 66 mgs. %, Culture and smear for organisms negative. Malaria smear: negative. Cold agglutinations. negative. Stool: negative for E. histolytica. Blood serum, taken for complement-fixation tests against lymphocytic choriomeningitis on the 22nd hospital day, was "negative."

The patient was well at the time of discharge of 4 July. Follow-up examinations done 2½ months after the acute illness, was negative for recurrence and residua. Serum was drawn at this time and tests performed. The tests and their results follow. Heterophile agglutination: negative. Lymphocytic choriomeningitis: complement-fixation and virus neutralization, both negative.

Case no. 4 (R. H.): Hosp. Reg. No. 14728. A 27 year old white male, admitted 7 June complaining of headache and some pain in the back of the neck.

P.H.: Non-contributory.

P.I.: Three days before admission the patient first noted a frontal headache which became increasingly severe and was accompanied by some pain in the back of the neck. The temperature became elevated on the morning of admission and some stiffness of the neck developed. There was no eyeache nor nausea, and no generalized aching.

P.E.: The patient had some nuchal rigidity and small glands were felt in the posterior triangle of the neck. No eyeball tenderness, nor pain on motion was found.

Course: A lumbar puncture was done on the second hospital day with some immediate relief of his symptoms. The temperature which had been elevated to 101 dropped and became normal on the third hospital day, remaining so thereafter.

Laboratory findings: Hgb., 90%. WBC, 11,550; seg., 78%; lym., 16%; mon., 1%; juv., 1%; eos., 1%. Urine: negative except for a rare RBC. Spinal fluid: 45 lymphocytes, Pandy negative, protein 38 mgs. %, sugar 69 mgs. %, culture and smear for organisms negative. Malaria smear: negative. Proteus agglutinations: OX19–1:20; OX2–0 OXK-O.

The patient was discharged on 1 July, having shown no residua of his infection. Examination after two months revealed no stigmata of the infection. He had a streptococcal throat infection at that time, so was readmitted to the hospital for treatment. A second lumbar puncture was done, which was entirely negative: Wbc-3 lymphocytes, Pandy negative, Protein-normal, Wassermann-negative. Serum was drawn at this time and tests performed. The tests and their results follow: Hererophile agglutination: 1:14. Lymphocytic choriomeningitis: complement-fixation and virus neutralization, both negative.

Case no. 5 (V. P.): Hosp. Reg. No. 14789. A 26 year old white male admitted 8 June complaining of severe headache.

P.H.: Non-contributory except for a mild diarrhea 1 month before admission and again 5 days before admission.

P.I.: The patient experienced frontal headache and pains in the eyes of three days' duration. He had one chill the day before admission.

P.E.: There was tenderness of the eyeballs and over the frontal sinuses. Small posterior cervical nodes were palpable, and there was some questionable nuchal rigidity.

Course: A lumbar puncture was done on the third hospital day, following which the headache decreased markedly, though the aching of the eyes still persisted.

The temperature was elevated to 102 the first day, but dropped to normal on the second day and stayed down. Symptoms had entirely disappeared by one week after admission.

Laboratory findings: Hgb., 10%. 8 June: WBC, 6,950; seg., 56%; lym., 39%; mon., 3%; eos., 2%. Spinal fluid: 217 all lymphocytes, 12 RBC's; Pandy trace, protein 58 mgs. %, sugar 71 mgs. %, culture and smear for organisms negative. Blood Kahn: negative. Malaria smear: negative.

The patient was discharged on 3 July having had no recurrence of symptoms and no residua of the infection. In the interval before his final check 2½ months after the acute illness, the patient had two more attacks of diarrhea comparable to the one just preceding the meningitis. Stool cultures and examination for E. histolytica were negative.

At the follow-up examination the patient showed no residua of the disease. Blood serum was drawn at this time and tests performed. The tests and their results follow: Heterophile agglutination: 1:28. Lymphocytic choriomeningitis: complement-fixation and virus neutralization, both negative. Japanese B encephalitis (Type B): virus neutralization negative.

Case no. 6 (P. M.): Hosp. Reg. No. 14865. A 23 year old white male, admitted 10 June complaining of a headache.

P.H.: Non-contributory.

P.I.: The patient noted a headache two evenings before admission, accompanied by a slight fever and some vomiting. He had had one or two extra stools for two days prior to admission, but no diarrhea.

P.E.: There was a mild acne about the shoulders. Minimal injection of the tonsils was noted with a few small posterior cervical lymph nodes bilaterally. No eyeball pain on motion nor tenderness could be elicited.

Course: A lumbar puncture was done on the second hospital day. The fever which had been elevated to 102 dropped to normal by the third hospital day and the headache disappeared on the same day. Ten days after admission the patient developed a furuncle of the arm which was treated with Sulfadiazine by mouth and local compresses. Complete recovery was made.

Laboratory findings: Hgb., 85%. 10 June: WBC, 11,050; seg., 64%; lym., 34%; mon., 2%. 28 June: WBC, 5,700; seg., 58%; lym., 34%; mon., 2%; eos., 6%. Urine: negative. Spinal fluid: WBC 49, all lymphocytes, RBC 19, Pandy trace, sugar 66 mgs %, culture and smear for organisms: negative. Cold agglutinations: negative. Stool: negative for E. histolytica. Blood serum taken on the 18th hospital day for complement-fixation tests against lymphocytic choriomeningitis was "anti-complementary."

The patient was discharged on 4 July having been well with no recurrence of meningeal symptoms. On 10 July he was readmitted to the hospital for a recurrence of headache of one day's duration. Physical examination was negative except for some slight general glandular enlargement and a minimal intention tremor. Blood and spinal fluid studies were negative, the latter showing: normal number of

cells, proteins, and negative pandy, negative Wassermann and gold curve. The headache cleared on the day after admission and the patient had no further recurrence nor residua.

A follow-up examination 2½ months after the original admittance revealed no further signs nor symptoms. Serum was taken at this time and tests performed. The tests and their results follow: Heterophile agglutination: 1:14. Lymphocytic choriomeningitis: complement-fixation and virus neutralization, both negative. Encephalitis, Western Nile type: virus neutralization negative. Encephalitis, St. Louis type: virus neutralization negative.

Case no. 7 (L. G.): Hosp. Reg. No. 14924. A 24 year old white male admitted 11 June complaining of severe frontal headache and pain in the eyes.

P.H.: Non-contributory.

P.I.: This began with a headache two days before admission to the hospital. The headache became more severe and frontal in character, and pain in both the eyes and neck was noted. This continued till admission.

P.E.: A few small tender glands were present in the posterior cervical chain. Some eyeball tenderness was noted, but no nuchal rigidity nor coryza.

Course: A lumbar puncture was done the day following admission. By three days after the puncture the patient had improved greatly and had no further symptoms. The temperature which had been elevated to 101, dropped to normal on the third day of hospitalization and remained normal thereafter.

Laboratory findings: Hgb., 85%. 12 June: WBC, 7,300; seg., 67%; lym., 30%; eos., 2%; juv., 1%. Urine: negative except for a rare WBC. Spinal fluid: WBC 33, 92% mononuclears, RBC 6, Pandy negative, protein 35 mgs. %, sugar 71 mgs. %, culture and smear for organisms-negative. Malaria smear: negative. Serum taken for complement-fixation tests against lymphocytic choriomeningitis on the 18th hospital day was reported "anti-complementary."

The patient had no recurrence of symptoms in the hospital and was discharged on 1 July. Examination two months post onset of the acute disease, was negative for any residua. Serum was again taken for lymphocytic choriomeningitis serologic tests and also for heterophile agglutinations. Heterophile agglutination: negative. Lymphocytic choriomeningitis: complement-fixation and virus neutralization, both negative.

Case no. 8 (D. G.): Hosp. Reg. No. 15123. A 19 year old white male, admitted 16 June complaining of headache and malaise.

P.H.: Non-contributory.

P.I.: The patient first noted a headache the afternoon before admission and during the night he had some aching of the back and joints, with beginning eyeache and fever. Some nausea was present and the headache became localized about the eyes.

P.E.: Small posterior cervical glands, eyeball tenderness and minimal nuchal rigidity were all present.

Course: A lumbar puncture was done the day after admission. Following this the frontal headache improved. The patient became ambulatory two days later, which seemed to provoke a return of the original symptoms. All symptoms disappeared by the eighth hospital day, the temperature having reached normal after six days.

Laboratory findings: Hgb, 90%. 16 June: WBC, 6,550; seg., 76%; stb., 3%; lym., 18%; mon., 3%. 10 June: WBC, 5,100; seg., 54%; stb., 2%; lym., 36%; mon., 8%. Urine: negative. Spinal fluid: WBC 3 lymphocytes, RBC, 1, Pandy 1, protein 60 mgs. %, sugar 73 mgs. %, culture and smear for organisms negative. Blood Kahn: negative. Blood smear: negative for parasites. Proteus agglutinations: negative. Stool: negative for E. histolytica. Blood serum was taken for complementary-fixation tests against lymphocytic choriomeningitis on the 17th hospital day. This was reported "anti-complementary."

The patient had no recurrence of symptoms and was well on discharge 18 July. A follow-up examination was not possible on this patient as he had left the environs of this hospital during the interval.

Case no. 9 (J. R.): Hosp. Reg. No. 15129. A 26 year old white male admitted 16 June complaining of headache.

P.H.: The patient drank untreated river water five days before admission.

P.I.: The patient experienced fever and generalized aching four days before admission. The night before admission he had chilly sensations, fever and severe frontal headache.

P.E.: Marked eyeball tenderness as well as pain on motion of the eyes was present. Slight nuchal rigidity was elicited and small posterior cervical glands were palpable.

Course: A lumbar puncture was performed the day after admission, following which the headache decreased in intensity. The temperature which ranged between 100 and 101 degrees rose one time to 103 degrees. This dropped to normal on the fourth hospital day. The patient became ambulatory the following day but because of a recurrence of the headache, was kept quiet until his tenth day when all symptoms had disappeared. Before discharge he had a mild two day diarrhea for which no causal agent was found.

Laboratory findings: Hgb., 90%. WBC, 5,250; seg., 50%; st., 13%; lym., 30%; mon., 5%; eos., 2%. Urine: negative. Spinal fluid: WBC 55, all lymphocytes RBC 10, Pandy negative, protein 67 mgs. %, sugar 52 mgs. %, culture and smear for organisms negative. Sedimentation rate: 20 mm./hr. Stool: negative for E. histolytica. Blood serum taken on the 26th hospital day for complement-fixation tests against lymphocytic choriomeningitis, was reported, "anti-complementary."

The patient had no further recurrence of his symptoms and was well at the time of discharge on 11 July. Follow-up studies done 2½ months after initial disease showed no residua. Serum was taken at this time and tests performed. The tests and their results follow. Heterophile agglutination. 1:14; Lymphocytic choriomeningitis, complement-fixation and virus neutralization, both negative.

Case no. 10 (O. S.): Hosp. Reg. No. 15134. A 25 year old white male admitted 16 June complaining of severe headache and nausea.

P.H.: Non-contributory.

P.I.: This began with severe frontal headache three days before admission. The following day a fever started with some nausea and vomiting, accompanied by aching in the back and chilliness.

P.E.: Tenderness of eyeballs, moderate nuchal rigidity and small posterior cervical glands were noted on admission.

Course: A lumbar puncture was done on the day following admission following which the headache decreased in intensity. The temperature, elevated to 99.8 degrees dropped to normal the day after the tap and remained normal thereafter. The symptoms disappeared by the fifth hospital day.

Laboratory findings: Hgb., 90%. WBC, 6,950; seg., 41%; lym., 50%; eos., 4%; bas., 1%; mon., 4%. Spinal fluid. WBC 45, 88% lymphocytes, RBC 1, Pandy negative, protein 39 mgs. %, sugar 66 mgs. %, smear and culture for organisms negative. Blood culture: negative. Blood smear: negative for parasites. Blood serum taken on the 13th hospital day for complement-fixation tests against lymphocytic choriomeningitis, was reported, "negative."

The patient had no recurrence of his original symptoms and was discharged on 1 July, but for a month following discharge he felt somewhat weak and tired with subsequent complete recovery. Studies 2½ months after the initial onset of the illness showed no residua. Serum was again taken for complement-fixation tests and also for a heterophile agglutination. Heterophile: positive at 1:224. Serum was taken at this time and tests performed. The tests and their results follow: Heterophile agglutination: 1:224. Lymphocytic choriomeningitis: complement-fixation, anti-complementary; virus neutralization, negative. Mumps: complement-fixation; negative.

Case no. 11 (R. V.): Hosp. Reg. No. 15135. A 31 year old white male, admitted 16 June complaining of severe frontal headache.

P.H.: Non-contributory.

P.I.: This began three days before admission with severe frontal headache accompanied by nausea and vomiting. In addition, the patient had some low back pain.

P.E.: There was a slight miliarial rash over the chest. Marked eyeball tenderness was present, with minimal nuchal rigidity and a few small posterior cervical lymph nodes.

Course: A lumbar puncture was done the day after admission with some diminution of the headache, but the latter remained present to some degree for five days after admission. His temperature was 99 degrees on admission and rose on one occasion to 100 degrees, but became normal by the fourth hospital day, and remained there.

Laboratory findings: Hgb., 90%. WBC, 5,800; seg., 58%; lym., 38%; eos., 2%; mon., 2%. Spinal fluid. WBC 45. 92% lymphocytes, RBC 12, protein 43 mgs. %,

sugar 63 mgs. %, culture and smear for organisms-negative. Sedimentation rate. 13 mm/hr. Blood culture: negative. Blood smear: negative for parasites. Blood serum taken for complement-fixation tests against lymphocytic choriomeningitis on the 14th hospital day was reported "negative."

The patient was completely well at time of discharge on 1 July. Follow-up studies some 2½ months after the initial disease showed no residua of the infection and no recurrence. Serum was taken at this time and tests performed. The tests and their results follow. Heterophile agglutination: 1:112. Lymphocytic choriomeningitis. complement-fixation and virus neutralization, both negative. Mumps: complement-fixation, negative.

Case no. 12 (J. P.): Hosp. Reg. No. 15173. A 21 year old white male admitted 17 June complaining of headache.

P.H.: Non-contributory.

P.I.: On the day prior to admission the patient developed some fever and a very severe headache. That night he had moderately severe vomiting and noted some pain in the back of the neck, following which the headache became frontal in location.

PR.: Some eyeball tenderness was noted as well as minimal nuchal rigidity. Small tender cervical lymph nodes were palpable bilaterally, in the posterior chain.

Course: The patient had a lumbar puncture done on the second hospital day with some relief of the headache. By the fourth hospital day the patient had recovered completely from his symptoms.

Laboratory findings: Hgb., 90%. WBC, 5,800; seg., 60%; lym., 29%; eos., 5% mon, 6%. Urine: trace of albumin and 3–5 WBC's H.P.F. Spinal fluid: WBC 197, 50% lymphocytes and 50% polymorphs (of the cells which could be identified, though many could not be, because of disintegration, Pandy: 1, protein. 57 mgs. %, sugar 72 mgs %, culture and smear for organisms negative. Stool: negative of E. histolytica.

The patient remained well after recovery from the acute symptoms and had no recurrence. He was discharged without residua on 7 July. No follow-up studies were done on this patient as he had left the environs of the hospital.

Case no. 13 (M. A.): Hosp. Reg. No. 15183. A 25 year old white male, admitted 21 June complaining of a headache.

P.H.: Non-contributory.

P.I.: Three days before admission, the patient developed symptomless fever 101–104 degrees. It subsided after one day. On the day before admission severe frontal headache was noted accompanied by sensitive eyeballs and some pains in the back of the neck.

P.E.: Marked eyeball tenderness, minimal nuchal rigidity and small posterior cervical nodes were present.

Course: A lumbar puncture was done on the day after admission with complete relief of the headache. There was no return of this symptom. The temperature

was 99.4 degrees on the day of admission and by the second day had dropped to normal. By the 3rd post admittance day the patient was well of all symptoms.

Laboratory findings: Hgb., 90%. WBC, 4,600; seg., 78%; lym., 18%; eos., 6%. Urine: negative except for a few RBCs. Spinal fluid: WBC 490, 80% polymorphs, 20% lymphocytes, Pandy 1, protein 70 mgs. %, sugar 62 mgs. %, culture and smear for organisms negative. Blood Kahn: negative. Blood smear: negative for parasites. Cold agglutinations: negative. Blood serum taken on the 14th day of hospitalization for complement-fixation tests against lymphocytic choriomeningitis was reported "anti-complementary."

The patient had no recurrence and was discharged as well on 1 July. Follow-up studies two months after the original illness revealed no residua of the disease. Serum was taken at this time and tests performed: the tests and their results follow: Heterophile agglutination: 1:224. Lymphocytic choriomeningitis: complement-fixation and virus neutralization, both negative. Encephalitis, Western Nile type; virus neutralization negative.

Case no. 14 (C. K.): Hosp. Reg. No. 15287. A 20 year old white male, admitted 20 June, complaining of aching and fever.

P.H.: Non-contributory.

P.I.: Two days before admission the patient developed a head cold and some aching of the eyes.

P.E.: Patient seemed moderately ill with some eyeball tenderness, a mild pharyngeal injection and palpable small posterior cervical glands. No nuchal rigidity.

Course: Moderately severe diarrhea developed on the second hospital day. Because of fever and the severity of the diarrhea, the patient was started on sulfadiazine, despite negative stool cultures. The temperature was 103 on the third hospital day and rose to 103.8 on the fourth day. The headache, which had been moderate and generalized until that time, became very severe and confined to the frontal region. A spinal puncture was done on the fifth day of hospitalization following which the headache was greatly relieved. The temperature thereafter began to decline, reaching normal on the eighth day of hospitalization. Signs and symptoms disappeared by the tenth day.

Laboratory findings: 21 June: Hgb., 95%; WBC, 13,700; seg., 73%; lymph., 27%. 24 June: Hgb., 85%; WBC, 9,100. 11 July: Hgb., 90%; WBC, 5,700; seg., 52%; lymph., 52%; mono., 4%. Urine: negative except for one plus albumin on first examination which later cleared. Spinal fluid: WBC 317, 99% ofwhich were lymphocytes, protein 60 mgs. %, Pandy one plus, sugar 61 mgs. %, smear and culture showed no organisms. Blood smear: no malaria parasites. Blood Kahn: negative. Heterophile agglutination: 1:7. Proteus agglutination: negative. Stool: microscopic examination negative; culture negative. Serum drawn on the 20th hospital day for complement-fixation against lymphocytic choriomeningitis was reported anti-complementary.

The patient was discharged on 21 July, having no recurrence of symptoms. Follow-up observation two months after the acute illness showed no residua. Serum

was taken at this time and tests performed. The tests and their results follow: Heterophile agglutination: 1:28. Lymphocytic choriomeningitis: complement-fixation negative. Encephalitis, St. Louis type; virus neutralization negative.

Case no. 15 (P. A.): Hosp. Reg. No. 15321. A 21 year old white male admitted 21 June complaining of frontal headache.

P.H.: Non-contributory.

P.I.: Patient had had a cold of two weeks duration which had subsided considerably at the time of admission. The day before admission he developed a slight headache which became worse and frontal in location.

P.E.: Marked eyeball tenderness, none found over the frontal sinuses. There minimal nuchal rigidity and small posterior cervical glands were palpable.

Course: A spinal puncture was performed on the second day of hospitalization, following which the headache nearly disappeared in several hours, and by the second post-puncture day the patient was symptomless. The temperature which had been elevated to 100.4 was normal by the third hospital day and remained normal.

Laboratory findings: Hgb., 90%. 22 June: WBC, 5,200; seg., 64%; stb., 11%; juv., 4%; lymph, 21%. Urine negative. C.S.F.: WBC 89, 84% lymphocytes, RBC 27, Protein 51 mgs. %, sugar 71 mgs. %, Pandy trace, culture and smear for organisms: negative. Brucella agglutination: negative. Proteus agglutination: negative. Heterophile agglutinations: positive 1:56 & 1:112. Stool: negative microscopic examination. The patient was discharged 10 July, having shown no recurrence of the symptoms of this disease. Follow-up studies two months after the acute illness revealed no residua. Serum was drawn at this time and tests performed. The tests and their results follow: Heterophile agglutination: 1:56. Lymphocytic choriomeningitis: complement-fixation and virus neutralization, both negative. Encephalitis, Japanese (Type B): virus neutralization, negative.

Case no. 16 (J. B.): Hosp. Reg. No. 15328. A 23 year old white male admitted on 21 June complained of fever and earache.

P.H.: About six weeks before admittance, the patient had had severe generalized aching, chills and fever with vomiting. At that time he was admitted to a hospital where he was delirious with a temperature as high as 106 degrees. He was treated for "meningitis" though no spinal puncture was done. He recovered and was discharged after two weeks, but since that time he had felt rather weak and tired.

P.I.: The patient developed a cold two days before admission with some mild frontal headache. With this he experienced some pains in the left ear.

P.E.: The patient was thin and looked more chronically than acutely ill. There was some tenderness below the left ear and a few small posterior cervical lymph nodes. Examination of the ear revealed otitis externa.

Course: Despite treatment the patient's ear became temporarily worse. On the third hospital day, however, it seemed to be subsiding, when the patient became more acutely ill, with severe frontal headache and slight meningismus. A purulent meningitis was considered unlikely as the patient was not that sick. Spinal punc-

ture was done with some relief of the severe headache. By the sixth hospital day the headache had completely subsided. The temperature which had been elevated to 101.8 degrees returned to normal on the sixth day also, which day marked the cessation of all acute symptoms. The patient needed continued treatment for the slowly clearing otitis externa until the 17th hospital day.

Laboratory findings: Hgb., 80%. 22 June: WBC, 7,500; seg., 66%; lymph. 30%; mon., 1%; stb., 3%. 24 June: WBC, 14,600; seg., 92%; lymph., 7%; eos., 1%. 27 June: WBC, 5,650; seg., 73%; lymph., 26%; eos., 1%. Urine: negative. C.S.F.: WBC 17, all lymphocytes, protein 32 mgs. %, Pandy negative, smear and culture for organisms negative. Malaria smear: negative. Proteus agglutination: negative. Heterophile agglutination positive 1:28. Stool: negative microscopic examination. Serum taken on the 18th hospital day for complement-fixation tests against lymphocytic choriomeningitis was reported: "anti-complementary."

The patient remained well after the ear infection subsided and he was discharged without recurrence on 29 July. Follow-up studies done two months after the acute illness failed to show any residua. Serum was drawn at this time and tests performed. The tests and their results follow: Heterophile agglutination: 1:112. Lymphocytic choriomeningitis: complement-fixation and virus neutralization, both negative. Encephalitis, Western Nile type: virus neutralization, negative. Encephalitis, St. Louis type; virus neutralization, negative.

Case no. 17 (J. F.): Hosp. Reg. No. 15490. A 25 year old white male admitted 25 June complaining of severe frontal headache.

P.H.: For one month prior to admission the patient had noted a slight malaise and tired feeling with anorexia, especially when working in the heat.

P.I.: Severe frontal headache was noted for three days before admission followed by some pain in the eye region.

P.E.: The patient appeared acutely ill with severe frontal headache. There was marked eyeball tenderness, slight nuchal rigidity to extreme forward flexion, and small posterior cervical glands were palpable bilaterally.

Course: A lumbar puncture was done the day after admission with some relief of the intensity of the headache. The temperature, elevated to 101 degrees, dropped to normal on the second hospital day and remained so. All symptoms had cleared by the fourth hospital day.

Laboratory findings: Hgb., 90%. 25 June: WBC, 9,100; seg., 67%; lymph., 19%; stb., 14%. 12 July: WBC, 4,500; seg., 73%; lymph., 26%; eos., 1%. Urine: negative, C.S.F.: WBC 4, all lymphocytes, protein 56 mgs. %, Pandy 1 plus, culture and smear for organisms; negative. Kahn: negative. Malaria smear: negative. Stool: Negative for E. histolytica. Serum taken for complement-fixation tests against lymphocytic choriomeningitis on the 14th hospital day was reported: "anti-complementary."

The patient was discharged on 16 July, having shown no recurrence of the original symptoms. Follow-up examination two months after the onset of the acute illness showed no symptoms. Serum was drawn at this time and tests per-

formed. The tests and their results follow: Heterophile agglutination: negative. Lymphocytic Choriomeningitis: complement-fixation and virus neutralization, both negative. Encephalitis, Western Nile type: virus neutralization, negative.

Case no. 18 (D. F.): Hosp. Reg. No. 15580. A 23 year old white male, admitted 28 June complaining of headache.

P.H.: Non-contributory.

P.I.: This began five days before admission with slight nausea and dome headache, for two days before admission he had had a slight fever with the headache which was severe especially in the frontal region and about the eyes.

P.E.: Marked eyeball tenderness and small slightly tender posterior cervical nodes were present. No nuchal rigidity and no coryza was noted.

Course: A lumbar puncture was done on the second hospital day with some relief of the intensity of the headache. The temperature, which at highest was 99 degrees was normal by the second day. All symptoms were gone by the third hospital day. The patient remained in the hospital for treatment of an amoebiasis infection.

Laboratory findings: Hgb., 85%. WBC, 4,350; seg., 53%; lymph., 45%; eos., 2%. Urine: negative. C.S.F.: WBC 40, 90% lymphocytes, protein: 50 mgs. %, Pandy: trace, culture and smear for organisms: negative. Blood Kahn: negative. Malaria smear: negative. Throat culture: normal flora with alpha streptococci. Blood serum taken for complement-fixation tests against lymphocytic choriomeningitis on the 14th hospital day was reported "negative."

The patient remained well throughout the rest of his hospitalization and was discharged on 27 August. Follow-up studies two months after the onset of the acute illness revealed no residua of the disease. Serum was drawn at this time and tests performed. The tests and their results follow: Heterophile agglutination: 1:14. Lymphocytic choriomeningitis: complement-fixation and virus neutralization both negative. Mumps: complement-fixation, negative.

Case no. 19 (F. H.): Hosp. Reg. No. 15617. A 23 year old white male, admitted 28 June, complained of a frontal headache and vomiting.

P.H.: Non-contributory.

P.I.: For two days before admission the patient had a headache which became increasingly severe. With this he had anorexia and some vomiting, for one day.

P.E.: Patient was moderately ill, but showed only posterior cervical lymphadenopathy. He had no eyeball tenderness and no nuchal rigidity.

Course: The severe frontal headache continued for three days, and with this, he vomited several times. He developed some pain in the eyes with motion. On the fourth hospital day he had a spinal puncture though his acute symptoms had subsided at that time.

Laboratory findings: 3 July: WBC, 9,700; seg., 81%; lymph., 15%; eosin., 3%. 10 July: WBC, 10,300; seg., 64%; lymph., 36%. Urine: an occasional WBC per high power field. Spinal fluid: WBC 84, all mononuclear cells, RBC 19, pro-

tein 36 mgs. %, chloride 735 mgs. % (as NaCl). Wassermann: negative. Gold curve: 0–0. Smear: no organisms seen. Culture: staphylococci (probably contaminant). Blood Kahn: negative. Blood smear: no parasites seen. Proteus agglutinations: OX19 1:20; OXK and OX2 negative. Stool examination: negative microscopically. Blood serum taken on the 16th day for complement-fixation against lymphocytic choriomeningitis was reported negative.

For about 2½ weeks after his acute infection the patient had some weakness and tired feeling which entirely disappeared at the time he was discharged on 2 August. Examination after 2 months showed no residua. Serum was drawn at this time and tests performed. The tests and their results are as follows: Heterophile agglutination: 1:7. Lymphocytic choriomeningitis: complement-fixation and virus neutralization, both negative. Mumps: complement-fixation, negative. Dengue: Hawaiian strain: virus neutralization, negative.

Case no. 20 (T. B.): Hosp. Reg. No. 15641. A 27 year old white male admitted 29 June complained of severe frontal headache.

P.H.: Non-contributory.

P.I.: Patient had a mild cold for one week; on the day of admission he developed severe frontal headache and some eye pain.

P.E.: Some nasal congestion and pharyngeal irritation were present. The neck was slightly stiff and there was small posterior cervical glands palpable bilaterally. The eyeballs were not tender.

Course: A lumbar puncture was done on the fourth hospital day after which the headache cleared considerably. The temperature which had been elevated to 100.5 returned to normal on the 3rd hospital day and remained so. Symptoms had entirely disappeared by the sixth hospital day.

Laboratory findings: Hgb., 85%; WBC, 7,300. 29 June: Seb., 78%; stab., 6%; lymph., 14%. 11 July: Seg., 48%; lymph., 44%; eosin., 6%; baso., 2%. Urine: negative. Spinal fluid: WBC 33, 74% of which were lymphocytes, protein 29 mgs. %, Pandy trace. Smear: fungi seen on one slide, probably a contaminant. Culture: staphlococci, probably contaminant. Stool examination: Negative microscopically. Blood serum was taken on the 16th hospital day for complement-fixation against lymphocytic choriomeningitis and was reported: negative.

Patient was discharged 22 July having no recurrence of symptoms. Follow-up examination done 2 months after the acute illness showed no residua from the disease. Serum was drawn at this time and tests performed. The tests and their results are as follows: Heterophile agglutination: negative. Lymphocytic choriomeningitis: complement-fixation and virus neutralization, both negative. Mumps: complement-fixation, negative. Encephalitis, Japanese (Type B): virus neutralization, negative.

Case no. 21 (F. A.): Hosp. Reg. No. 15647. A 24 year white male admitted 29 June complaining of headache and back-ache.

P.H.: Non-contributory.

P.I.: Five days before admission patient began to experience some backache. Two days later a frontal headache began, followed by chilly sensations and a mild cough.

P.E.: There was some eyeball tenderness and conjunctival injection. Small posterior cervical glands were present bilaterally, no nuchal rigidity.

Course: Some improvement was noted after two hospital days. Severe frontal headache persisted however. A lumbar puncture was done on the fifth hospital day. The headache persisted for less than one day following the procedure. The temperature which had been elevated as high as 104.2 dropped to normal on the fifth day and remained so.

Laboratory findings: Hgb., 85%. 29 June: WBC, 9,900; seg., 72%; lymph., 22%; mono., 2%; eosin., 4%. 11 July: WBC, 6,900; seg., 58%; lymph., 38%; mono., 2%; eosin., 2%. Urine: negative. Spinal fluid: 220 lymphocytes, protein 55 mgs. %, sugar 63%, Chlorides 700 mgs. % (as NaCl). Wassermann: negative 0.1 cc., positive 0.5 cc. Gold curve: 0–0. Blood Kahn: negative. Blood smear: no parasites seen. Stool examination: microscopically negative. Blood serum was taken for complement-fixation against lymphocytic choriomeningitis on the 14th hospital day, reported: negative. Serum was also run against the Hawaiian strain of Dengue and no neutralizing antibodies found. Patient was asymptomatic at the time of discharge 20 July.

Follow-up examination 2 months after the onset of the acute illness revealed no residua. However, two weeks later, patient was admitted again to the hospital for "fever of unknown origin." He had headache and back ache on this occasion also, physical exam revealed no abnormality beyond conjunctival injection. A lumbar puncture at this time revealed entirely normal spinal fluid. His symptoms and fever disappeared after four days of hospitalization. No diagnosis was made. Serum was drawn 2 months after the onset of original disease and tests performed. The tests and their results follow: Heterophile agglutination: negative. Lymphocytic choriomeningitis: complement fixation and virus neutralization, both negative. Encephalitis, Japanese (Type B): virus neutralization, negative.

Case no. 22 (C. G.): Hosp. Reg. No. 15670. A 19 year old white male admitted 29 June complaining of severe headache.

P.H.: Non-contributory.

P.I.: The night before admission patient experienced a severe frontal headache radiating to the supra-orbital area and into the eyes. He had one mild attack of vomiting.

P.E.: Patient appeared moderately ill. He had definite eyeball tenderness, slight nuchal rigidity and a few small posterior cervical lymph nodes bilaterally.

Course: A lumbar puncture was done the day after admission with some relief of the headache. The temperature, which had been elevated to 100.4 dropped to normal by the 3rd hospital day and remained there. The headache and other symptoms had entirely disappeared by the end of the 3rd hospital day and the patient remained well thereafter.

Laboratory findings: Hgb., 85%. WBC, 9,600; seg., 87%; stab., 3%; lymph., 6%; mono., 4%. Urine: negative, except for rare RBC per high power field. Spinal fluid: WBC 44, 84% lymphocytes, RBC 5, protein 39 mgs. %, sugar 72 mgs. %, Pandy negative, chlorides 770 mgs. % (as NaCl). Wassermann: positive 1.0 cc. Gold curve: 0–0. Smear: negative. Culture: grampositive bacillus, probably a contaminant. Blood Kahn: negative. Blood smear: no parasites seen Stool examination: Giardia lamblia seen. Blood serum taken on the 17th hospital day for complement-fixation against lymphocytic choriomeningitis, reported: negative.

Patient discharged without residua on 21 July. Follow-up examination 2 months after the acute illness showed no residua. Serum was drawn at this time and the tests performed. The tests and their results are as follows: Heterophile agglutination: 1:7. Lymphocytic choriomeningitis: complement-fixation, anticomplementary. Mumps: complement-fixation, negative.

Case no. 23 (R. M.): Hosp. Reg. No. 15675. A 27 year old white male admitted 30 June complaining of a cold with a cough.

P.H.: Non-contributory.

P.I.: Patient had a chronic non-productive cough for one month. For one week his upper respiratory symptoms increased. Two days before admission he developed a headache which became very severe just before admission.

P.E.: A mild pharyngitis was noted, with a few small posterior cervical lymph glands. Eyeball tenderness was present, but no nuchal rigidity. A few ronchi were heard in the chest.

Course: The headache became increasingly severe and on the 3rd day a suggestion of nuchal rigidity was present. A lumbar puncture was done on the 4th hospital day with some relief of the headache the following day. The temperature remained elevated 4 days reaching a high of 101.2. The headache persisted for 6 hospital days in all after which time all symptoms subsided.

Laboratory findings: Hgb., 90%. 1 July: WBC, 10,800; seg., 73%; stab., 2%; lymph., 25%. 12 July: WBC, 8,300; seg., 40%; juv., 1%; lymph., 59%. Urine: negative. Spinal fluid: WBC 283, 94% lymphocytes, Protein 42 mgs.%. Blood smear: no parasites seen. Stool examination: microscopically negative. Blood serum was taken on the 14th hospital day for complement-fixation against lymphocytic choriomeningitis. This was reported: negative.

Follow-up examination 2 months after the acute illness revealed no residua. Serum was drawn at this time and tests performed. The tests and their results follow: Heterophile agglutination: negative. Lymphocytic choriomeningitis: complement fixation and virus neutralization, both negative. Mumps: complement-fixation, negative.

BIBLIOGRAPHY

1. ARMSTRONG, C.: Mil. Surgeon, 1942, **91**, 129.
2. BLACKFAN, K. D.: Virus and Rickettsial Dis., A Symposium. Harvard Univ. Press, 1943.

3. Cullinan, E. R., and Whittaker, S. R. F.: Brit. Med. Journ., 1943, **2**, 543.
4. Eaton, M. D., Murphy, W. D., and Hanford, V. L.: Journ. Exp. Med., 1944, **79**, 539.
5. Farmer, T. W., and Janeway, C. A.: Medicine, 1942, **21**, 1.
6. Glendy, R. E., Beaser, S. B., and Hankins, W. D.: Arch. Int. Med., 1945, **75**, 30.
7. Kisner, P., and Lisansky, E. T.: Ann. Int. Med., 1944, **20**, 41.
8. LeGac, P., Samara, M., and Servant, J.: Bull. Soc. path. exot., 1939, **32**, 473.
9. Mackie, Hunter and Worth: Manual of Trop. Med., Natl. Res. Council, Saunders, 1945.
10. Pearson, A. S.: Brit. Med. Journ., 1941, **2**, 303.
11. Rogers and Megaw: Tropical Medicine. 4th ed., J. & A. Churchill, Ltd., London, 1942.
12. Rogers, A. M., and Elsom, K. A.: Field Med. Bull., U. S. F., I. B. T., 1945, **4**, 316.
13. Sabin, Albert B., Philip, C. B., and Paul, J. R.: J. A. M. A., 1944, **125**, 603, 693.
14. Simmons, J. S.: Virus and Rickettsial Dis., A Symposium, Harvard Univ. Press, 1943.
15. Wintrobe, M. M.: Clinical Hematology. Lea & Febiger, 1942.

STUDIES ON SCHISTOSOMIASIS JAPONICA

2. Analysis of 364 Cases of Acute Schistosomiasis with Report of Results of Treatment with Fuadin in 184 Cases

LT. COL. WALTER L. WINKENWERDER, MC, CAPT. ARNE V. HUNNINEN, SN.C., CAPT. THURSTON HARRISON, MC, MAJOR F. TREMAINS BILLINGS, MC, CAPT. DOUGLAS G. CARROLL, MC, AND CAPT. JOHN MAIER, MC.

From the 118th General Hospital, United States Army

I. INTRODUCTION

The epidemic of acute schistosomiasis japonica, which began soon after the reoccupation of Leyte, P. I. on the 20th of October, 1944 terminated in May 1945. The last case on which this diagnosis was made in this hospital was admitted in May.[1] Several studies of this epidemic have already been reported (1, 2), including three from this hospital (3, 4 and 6). One of these (3) presented a clinical study of 337 patients observed between late December 1944 and May 1945. That report which supplemented the first publication on this epidemic by Thomas and Gage (1), discussed the clinical aspects of the acute phase of tbe disease and presented data on exposure, latent period, symptomotology, physical findings and eosinophilia. In addition, a modification of the sedimentation method for concentrating ova inthe stools was described. Finally, the results of treatment with faudin in 110 cases observed for several weeks after the completion of treatment, was included.

The purpose of the present report is to review and summarize the observations made at the 118th General Hospital. This may involve some repetition, but it should be pointed out that an acute outbreak of schistosomiasis japonica of the magnitude which occurred on Leyte island has never heretofore been recorded, and may never occur again. The medical officers concerned have had the unique opportunity to watch the epidemic develop, to observe and eventually, to recognize the variable clinical manifestation of the initial illness, and further, to follow many of the cases for several months after treatment.

[1] During the preparation of this manuscript one additional case was admitted during early August 1945; exposure occurred when the individual fell out of a boat into fresh water early in July.

Comments will be made on the development and decline of the epidemic, on the severity of the symptoms which the disease produced, and on the eosinophilia which has been considered so characteristic, and upon which so much diagnostic emphasis has been placed. In a small number of patients, the laboratory diagnosis prior to treatment was based on the presence of only immature ova of *S. japonicum*[2] in the stools; however, mature ova were subsequently found in all of these patients. It is now known that in a few cases having characteristic symptoms and eosinophilia, it may be impossible to find ova in the stools even after repeated careful examinations extending over a period of weeks and even months after the onset of symptoms. A few such cases observed during the epidemic will be described. Observations have also been made upon the effects of chemotherapy on ova in the stools. Finally, post-treatment observations on cases treated with 40 cc. of fuadin will supplement the report contained in our first study (3).

II. GENERAL CONSIDERATIONS

A total of 364 cases of schistosomiasis japonica were observed in this hospital. 352 were of the white race, and 12 of the colored race. 362 cases developed the disease as a result of exposure to infested water on the island of Leyte. 2 cases presumably acquired the disease on Luzon as they gave no record of residence on other islands of the Philippine archipelago. Although cases of schistosomiasis japonica have never been traced to Luzon, and the intermediate snail host, Oncomelania quadrasi, has not been found in surveys, the authors have no further information regarding sources of infection in these two cases. On the other hand, Mindoro, Samar and Mindanao are recognized endemic areas yet no cases in which the disease could be definitely traced to these islands have come to our attention.

III. DEVELOPMENT OF THE EPIDEMIC

Exposures to fresh water infested with cercariae of *S. japonicum* occurred immediately following the reoccupation of Leyte on the 20th of October 1944 (5). Between the 20th and the 30th of November, the

[2] Criteria for recognition and identification of immature ova of S. japonicum were given in a previous paper (3).

first four cases in this series developed symptoms[3] considered as characteristic of schistosomiasis. As indicated in Table I, the epidemic developed rapidly, reached its peak in January, and then subsided gradually. The last case to be observed developed symptoms in May, the 7th month of the epidemic. Table II records the severity of symptoms as observed in 352 cases. The asymptomatic cases were admitted as suspect cases following surveys of organizations in which active cases were occurring, or they were picked up on routine stool examinations in the hospital while under observation for another illness. To illustrate how the epidemic expressed itself in terms of symptoms, the data in

TABLE I

Schistosomiasis Japonica—Monthly Incidence of Development of Symptoms in 348 Cases

Asymptomatic cases are included in month in which stools were found positive for ova of S. japonicum (see Table III). In 16 cases date of onset not determinable.

NOV.	DEC.	JAN.	FEB.	MAR.	APR.	MAY
4	80	155	74	24	8	3

TABLE II

Schistosomiasis Japonica—Severity of Symptoms as Observed in 352 Cases

In 12 cases data were not available for tabulation.

SEVERE	MODERATELY SEVERE	MILD	ASYMPTOMATIC
30 (8.5%)	110 (31.2%)	187 (50.3%)	25 (10%)

Tables I and II have been combined in Table III. Of the 4 cases developing symptoms in November, 2 were considered as moderately severe and 2 mild. In December there was a definite preponderance of the severe and the moderately severe cases. Thereafter, the mild cases predominated as the epidemic waned. In February, the fourth month of the epidemic, the first of the asymptomatic cases were seen.

Considering the average length of the latent period, namely, about 40 days in the severe and 50 days in the mild cases, with extremes from

[3] A detailed description of the symptoms characterizing the initial illness was given in previous papers (1, 3). Briefly, in order of frequency, they were fever, headache, stiff neck, malaise, loss of weight, anorexia, upper abdominal pain, especially, in the epigastric region, abdominal cramps, constipation and diarrhea.

26 to 58 days, the majority of exposures occurred during October, November and December when combat troops and even service personnel of necessity had to enter fresh water. By January, rigid con-

TABLE III

Schistosomiasis japonica—Month of Onset of Symptoms Correlated with Severity of Symptoms in 348 Cases

The epidemic covered a period of six months. The great majority of severe and moderately severe cases developed during the first three months, and exceeded the mild cases in the first two months; thereafter, the mild and asymptomatic cases, appearing in February, predominated. Onset in Asymptomatic group corresponds to date stools were found positive for ova of S. japonicum. (N-) represents number of cases with neurologic disturbances. In 14 cases data were not available for tabulation.

SEVERITY OF SYMPTOMS	NOV.	DEC.	JAN.	FEB.	MAR.	APR.	MAY	TOTAL CASES
Sev.		13(N-2)	12(N-1)	2(N-1)	1	1(N)	1(N)	30
M.S.	2	36	53(N-2)	15	3	0	1	110
M.	2	31	90(N-3)	48	11	1	0	183
A.				9	9	6	1	25
Total.....	4	80	155	74	24	8	3	348

TABLE IV

Schistosomiasis japonica—Comparing Month of Onset of Symptoms (Table III) with Month in Which Diagnosis Was Made by Finding of Ova in Stools in 348 Cases

Upper row of figures represents month of onset of symptoms. Lower row of figures represents month in which stools are found positive for ova. Note the lag between onset and diagnosis; this is more evident in mild cases and is due to delay in reporting to hospital and to difficulty in finding ova in some cases.

In 16 cases data not available for analysis.

SYMPTOMS	NOV.	DEC.	JAN.	FEB.	MAR.	APR.	MAY
Severe		13	12	2	1	1	1
			5	20	2	1	2
Mod. severe	2	36	53	15	3	0	1
			32	64	12	1	1
Mild	2	31	90	48	11	1	0
			6	96	58	6	1
Asymptomatic				9	9	6	1
				9	9	6	1

trol measures instituted as quickly as the tactical situation permitted, brought about a sharp decline in the incidence of the disease, although mild and asymptomatic cases continued to appear during March and April. The continued appearance of the mild cases was due in part to the longer latent period, and in part to the fact that many of the mild cases continued to remain on duty, despite their symptoms. The lag between exposure and diagnosis is illustrated in Table IV. This lag includes the delay in reporting to the hospital after symptoms developed, and also the time required to make the diagnosis. It is particularly prolonged in the mild cases due in part to the difficulty in finding ova: for example, in two mild cases developing symptoms in the latter part of November, the diagnosis was not made until 30 January in one, and 2 February in the other.

IV. LABORATORY DIAGNOSIS OF SCHISTOSOMIASIS JAPONICA

The laboratory diagnosis prior to treatment was based on the finding of mature ova in 344 cases (93.4 per cent) and immature ova in 16 cases (6.6 per cent), as shown in Table V. In 7 cases, to be discussed later, ova were not found prior to treatment, and the diagnosis was based on clinical symptoms and eosinophilia. In the majority of cases in which mature ova were found, immature ova were also present (see Table IX), but in some of the moderately severe and in some of the mild and asymptomatic cases, the initial diagnosis was based on the finding of immature ova alone. Later, during the course of treatment or after treatment was completed, mature ova were found. The following case report in which only immature ova were found prior to treatment is instructive and also indicates the paucity of ova in the stools of some cases while manifesting characteristically severe symptoms of the disease. Case 131, (Chart 3), a hospital corpsman was exposed to infested water in late November 1944. On 8 January 1945 symptoms of moderate severity developed. The fever was remittent, the liver enlarged and tender, and the spleen palpable. During the acute phase, transient jaundice and pulmonary infiltration were noted. The leucocyte count was 35,000 with 70 per cent eosinophiles. The laboratory reported "probable" immature schistosome ova on the 10th stool examination. Because of the severe symptoms, so characteristic of schistosomiasis, it was considered wise not to withhold treatment.

Improvement followed chemotherapy (40 cc. fuadin) and he was discharged to duty. In the 5th and 6th post-treatment week, immature ova were found, at which time transient fever, headache, and malaise were complained of. From the 9th to the 18th week inclusive, 7 stools done at approximately weekly intervals, were negative. In the 11th

TABLE V

Schistosomiasis japonica—Results of Original Diagnostic Stool Examinations in 367 Cases

In the 16 cases in which the laboratory diagnosis was based on immature ova only, mature ova were eventually found. In 7 cases diagnosis was made on basis of known exposure to fresh water suspected of being infested with cerceriae of S. japonicum, and on clinical symptoms and eosinophilia. (See Sect. V for discussion of this group.)

MATURE OVA	IMMATURE OVA	NO OVA
344 (93.4%)	16 (4.3%)	7* (2.3%)

* Includes 3 cases in which clinical diagnosis was not confirmed; ova had not been found during period of this study.

TABLE VI

Schistosomiasis japonica—Incidence of Eosinophilia of Blood Correlated with Severity of Symptoms in 352 Cases

Absence of eosinophilia occurred in 28 (8%); note that this also occurs in the severe groups, but is higher in the mild and asymptomatic cases.

SEVERITY OF SYMPTOMS	NO. OF CASES	EOSINOPHILIA	
		Positive	Negative
Severe	30	27 (90%)	3 (10%)
Mod. severe	110	106 (96.3%)	3 (3.9%)
Mild	187	170 (90.9%)	16 (9.0%)
Asymptomatic	25	19 (76%)	6 (24%)
Totals	352*	322 (92%)	28 (8%)

* In 12 cases data were not available for correlation.

and again in the 19th week, sigmoidoscopic examination revealed intestinal nodules, as described by Johnson and Berry (2). Finally in the 20th week, coincident with another attack of transient fever and mild symptoms, one mature ovum was found. Three days later numerous mature ova appeared in the stools. Throughout this period the white blood cells and eosinophiles remained high. The liver and spleen al-

though palpable were not much enlarged. The former was slightly tender. The patient was given a second course of treatment.

An eosinophilia[4] of the blood was found in 322 cases and was absent in 28 cases, on which data are available (Table VI). The eosinophilia during the initial illness was described in detail in our previous report. Counts of from 10,000 to 50,000 total white blood cells, with percentages for eosinophiles ranging from 10 to as high as 85 per cent were common. There was also an increase in the neutrophilic leucocytes. Charts 1 and 2, to be discussed later, give the pre- and post-treatment total white blood cell values and percentages of eosinophiles in a group of patients treated with fuadin. They may be referred to as representative of the entire series.

Emphasis should be placed on the absence of eosinophilia in an appreciable number of cases. Though this was more frequent in the mild and asymptomatic cases, the lack of an eosinophilic response in the blood was also noted in a few cases with severe symptoms. It is obvious therefore that absence of eosinophilia does not exclude schistosomiasis.

twas stated in our previous report that the eosinophilic response of the blood was unrelated to the severity of symptoms. This statement was based on a study of the first 75 cases seen at the height of the epidemic, when the more severe cases predominated. As Table VI shows, this statement does not now apply; the eosinophilic response is somewhat less in the mild and asymptomatic cases.

V. THE DIAGNOSIS OF SCHISTOSOMIASIS JAPONICA IN THE ABSENCE OF SCHISTOSOME OVA

The clinical diagnosis of schistosomiasis in the absence of ova was discussed in a previous report (3), when 3 such cases were described. Since then 4 additional cases have been added. In an individual presenting typical symptoms, a history of exposure and an eosinophilia with or without enlargement of the liver and spleen, the diagnosis is usually clear, at least in an endemic area. The data in these 7 cases are summarized in Table VII. In 4 cases the symptoms were mild, in the other 3 moderately severe. Hookworm ova were found in 3 cases. Eosinophilia was present in each case except in Case 330, in which

[4] In excess of 500 eosinophiles per cubic millimeter of blood.

however, the symptoms were typical and following chemotherapy, the eosinophilia increased and intestinal nodules were found on sigmoidoscopic examination. It was also possible to make post-treatment ob-

TABLE VII

Schistosomiasis japonica—Clinical Diagnosis—Stools Negative for Ova of S. Japonicum

Diagnosis was made on basis of history of exposure, symptoms and eosinophilia. Note that 2 cases (Nos. 281, 289) eventually showed ova of S. japonicum.

CASE NO.	ONSET AFTER	SEVERITY OF SYMPTOMS	STOOL EXAMINATION		WBC AND EOSIN. (×1000)		SIGMOIDOSCOPIC EXAMINATION (POST-TREATMENT)
			Before treatment	After treatment	Before treatment	After treatment	
40	Hepatitis	Mild	0	A.L. (7) Str.	9 × 27	11 × 9	0(10)
206		Mild	H.W.	H.W. (2) *IM.	11.8 × 23	14 × 17	0(7)
281		Mod. severe	H.W.	H.W. (14) S.J.	23.4 × 63	15.6 × 31	+(14)
289		Mod. severe	0	S.J. (5) IM.	Eosin.+	—	—
330		Mod. severe	H.W.	0 (17)	7.7 × 6	9.7 × 14	?+(11)
440	Malaria	Mod. severe	0	IM. (9)	6 × 40	6 × 12	—
444		Mild (N)	0	H.W. (2) Oxy	14 × 11	7.7 × 7	—

* In 2 other cases (Nos. 206, 440) immature ova were reported, substantiating but not verifying diagnosis. See Sect. V for discussion. Numerals in parenthesis indicate weeks after treatment when last examinations were made.

Abbreviations:

S.J.—Mature ova.
IM.—Immature ova.
H.W.—Hookworm ova.
A.L.—Ascaris ova.
Str.—Strongyloides ova.
Oxy.—Oxyuris Vermicularis ova.
(N)—Neurologic involvement.
— —No data.

servations on the other 6 cases. In Cases 281 and 289[5] mature ova of S. japonicum were found; in Case 281, intestinal nodules were found as

[5] In Cases 281 and 289, a diagnosis of relapse was made. See Sect. VIII and Chart 1.

well as ova. In Cases 206 and 440 immature ova were reported. In 2 cases, 40 and 444, the diagnosis in retrospect seems questionable, since there is as yet no confirmatory evidence. In Case 444, the neurological lesion was manifested by weakness in the right arm and shoulder girdle, and did not conform to the other neurological disturbances seen in this series (6).

TABLE VIII

Schistosomiasis japonica—Chemotherapy Employed in 364 Cases

Tartar Emetic in 5 cases and Anthiomaline in 5 cases produced reactions sufficiently severe to necessitate shift to fuadin. Results of treatment with 40 cc. fuadin are analyzed in Sect. VIII. Results of treatment with larger amounts of fuadin and other drugs will be reported later.

DRUG	CC.	GMS. ANTIMONY	NO. OF CASES
Fuadin	40	0.340	210
Fuadin	60	0.510	14
Fuadin	65	0.552	1
Fuadin	70	0.595	1
Fuadin	75	0.638	1
Fuadin	80	0.680	38
Tartar emetic†		0.680	78
Tartar Emetic and fuadin*			5
Anthiomaline	68	0.680	11
Anthiomaline and fuadin*			5

* Relative amounts of each drug varied slightly in each case; exact dosage will be included in a later report on results of treatment with these drugs.

† Includes 6 cases transferred to this hospital for observation following diagnosis and treatment.

VI. CHEMOTHERAPY

Table VIII summarizes the amount and type of chemotherapeutic agents used. 210 patients were treated with 40 cc. of fuadin (0.34 gms. Antimony). Availability, ease of administration and the low incidence of reactions prompted the use of this drug during the first two months of the epidemic. In February, however, a series of 77 cases was started on tartar emetic, of which 72 received a total of 0.68 gms. Antimony. In 5 patients, reactions occurred and necessitated a shift to fuadin for completion of treatment. In March when the first relapses following treatment with 40 cc. of fuadin were noted, schedules calling for 60 and

80 cc. were started. A small supply of anthiomaline became available in March and an additional small group of patients was treated with this drug. As relapses following 40 cc. of fuadin continued to develop retreatment with 60 cc. of fuadin given in doses of 5 cc. daily for 12 days was instituted. No reactions were observed.

The results of treatment with 40 cc. of fuadin will be discussed below; the results obtained with larger amounts of fuadin, with tartar emetic and anthiomaline will be reported in a subsequent paper. It may be stated at this time, however, that relapses have followed each treatment schedule described above.

VII. EFFECT OF CHEMOTHERAPY ON OVA IN THE SCHOOL

During the height of the epidemic when unusual demands were being made on the laboratory, it was impossible to examine stools daily during and after treatment. In many cases, however, stool examinations were made twice weekly, and the results soon indicated that ova disappeared during or soon after treatment was completed, and did not reappear for several weeks. The earliest reappearance of ova was in the 4th post-treatment week (see Sect. VIII (b) and Chart 1). More recently, it has been possible to examine stools daily in 24 cases. It was desirable to determine how long ova persisted in the stools, the variation in the daily output if any, the ratio of mature to immature ova, and finally, to determine whether the viability of ova was affected by chemotherapy.

The sedimentation method for concentrating ova, used throughout this study, has been described previously (3). Approximately 7 grams of stool was emulsified and sedimented for each examination, and a final sample covering $\frac{3}{4}$ of a microscope slide was examined thoroughly. The exact number of mature and immature ova was counted. Only the yellow colored ova which were *apparently* alive were included as representing the immature forms. A fully developed miracidium was present in all the mature eggs. Counts were not made of the dark, degenerating or unfertilized ova.

The data on 16 representative cases are recorded in Table IX. Each of these cases received 60 cc. of fuadin in doses of 5 cc. daily.

Reference to Table IX shows that ova were found in stools for from 9 to 16 days after the first day of treatment. The average was 13 days.

Examinations, not recorded in the table, show that the stools, when examined at frequent intervals, continued to be negative, except in

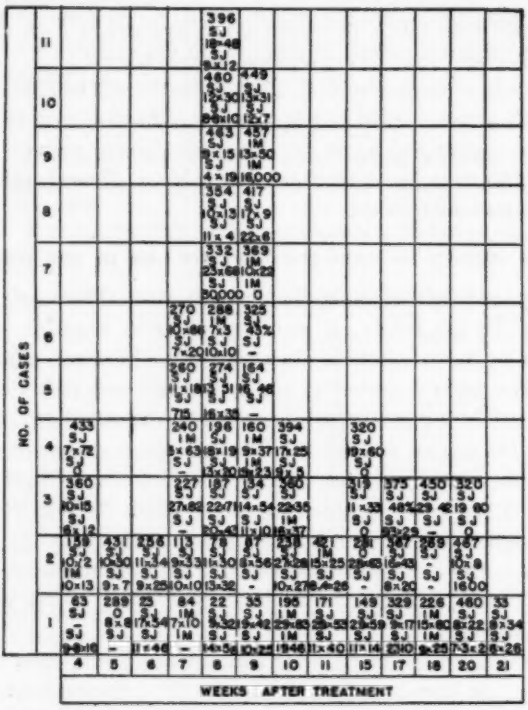


CHART 1. SCHISTOSOMIASIS JAPONICA—RELAPSES IN 55 CASES FOLLOWING TREATMENT WITH 40 CC. (0.340 GMS ANTIMONY) FUADIN

Each square represents 1 case. Reading from above downward is the case number, result of pre-treatment stool examination, the pre-treatment white blood cell count (× 1,000) and percentage of eosinophiles (× 1,000), the post-treatment stool and blood examination. S.J.—mature ova; I.M.—immature ova; —indicates no data; 0—negative; %—eosinophilia. In cases 260, 332, 457, 329, and 467, total eosinophilic values are given. Note that the majority of relapses occurred during the 7th, 8th, and 9th weeks.

Case 3, in which ova reappeared on the 80th post-treatment day. The table also shows that the number of ova, both immature and mature,

found in the random sample of stool remains fairly uniform from day to day. The total number of ova counted in the 24 cases was 1651. 260 or 14 per cent were immature forms. The percentage of immature ova varied from none to as high as 23 per cent of the total number of ova counted. Large numbers of ova were found in Cases 3 and 401, previously untreated cases; and in Cases 23 and 460, which were being treated for their first relapse. In the remaining cases, a smaller but relatively constant number was found. This indicates that the ova were fairly evenly distributed in the random stool specimens. The stools were all formed and mucus and blood were not seen.

Active miracidia were found in most of the mature ova before, during and after treatment. This indicates that the viability of the ova was unaffected by chemotherapy, which confirms Vogel's (7) experiments in the animal. Immature ova were also found up to the last day that eggs could be detected. The yolk was rich yellow in appearance, and the ova appeared to be viable. Since they had not developed to maturity, some factor may have inhibited this process, but this factor is not related to chemotherapy, as they were found before and after treatment in this experiment as in other cases in this series.

The viable eggs found in our cases disappeared from the stools, approximately within the period required for maturation of the egg. It would appear therefore, that the antimony therapy affects either ovulation or the deposition of new eggs in the wall of the intestine. Fairley (8) over 20 years ago found that chemotherapy inhibited ovulation in the schistosome. Chemotherapeutic experiments in animals, carried out by Bang and Hairston (9) at this hospital, confirm Fairley's observation as far as *S. japonicum* is concerned. The results noted in the stool examinations on patients can best be explained by the effect of the therapeutic agent upon ovulation.

VIII. POST-TREATMENT OBSERVATIONS ON CASES TREATED WITH 40 CC. OF FAUDIN

210 cases were treated with 40 cc. of fuadin according to the following schedule: 1.5 cc. on the first day, 3.5 cc. on the 3rd day, and 5 cc. on alternate days thereafter. This group includes only those cases in which treatment with fuadin was started prior to the middle of March. In a recent report from this hospital, a brief summary of the results of

TABLE IX

Effect of chemotherapy (fuadin) on presence of ova in stools

Table illustrates the day following institution of treatment on which viable ova can no longer be found in stools. Upper figures represent actual number of immature, and lower figures number of mature ova found on one entire glass slide. Cases 3 and 401 represent new, previously untreated cases, remaining cases represent first relapse, except for Cases 325 and 369 which were studied during second relapse (see Table XVI). Note that ova disappear at about same time in all three groups. Note that number of ova, both immature and mature, remains fairly uniform from day to day.

CASE NO.	DAYS PRIOR TO TREATMENT							DAYS AFTER INSTITUTION OF TREATMENT																				LAST DAY OVA FOUND
								Fuadin 5 cc. daily—12 days											Post-treatment										
	7	6	5	4	3	2	1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
23	1	0	0	0	6	6	6	4		5	10			2		6			6		6			0		0	0			13
	45	30	28	24	11	24	20	10		28	28			18		15			34		34			0		0	0			
401								3	2	2		8	4						2	5		3		0		0		0		14
								56	40	50		22	11						0	15		4		0		0		0		
3								1			3	0	6		0	21	22						3					0		15
								18			31	0	54		18	69	23						0					0		
460								2	4		4	0		3		1				0	0	0								12
								15	18		37	5		47		40				4	0	0								
319				0	1				0			0	1			2	0	0				2	0			0	0		0	14
				3	4				7			3	1			5	3	2				2	0			0	0		0	
145	0	0	0	0			0				0	1			1	0	1			0		0	0				0		12
	2	0	2	0			3				2	3			0	1	2			2		0	0				0		

| | | |

treatment in 110 cases of this group was given. They are included in the present analysis.

(*a*) *Clinical Observations:* Table X correlates the eosinophilia of the blood with the severity of symptoms in 203 of the 210 cases. This group includes 4 of the severe, 4 of the asymptomatic cases, and 2 in which the diagnosis was made clinically and ova were eventually detected. Compared to the series as a whole, the percentages for eosinophilia are essentially the same.

TABLE X

Schistosomiasis japonica—Series Treated with 40 cc. of Fuadin (0.340 gms. Antimony)—Eosinophilia Correlated with Severity of Symptoms in 203 Cases

Only a small number of the severe and asymptomatic cases are in this group. The incidence in this group of eosinophilia is about same as for entire series. Data on 7 cases not available for study.

SEVERITY OF SYMPTOMS	NO. OF CASES	EOSINOPHILIA	
		Positive	Negative
Severe	4	4 (100%)	0
Mod. severe	77*	74 (94.8%)	3 (5.2%)
Mild	118	108 (91.5%)	10 (8.5%)
Asymptomatic	4	3 (75%)	1 (25%)
Totals	203	189 (93.1%)	14 (6.9%)

* Includes 2 cases in which stools were negative prior to treatment.

It is pertinent now to recall the classification of cases, which was originally applied in our first report (3). Cases with severe symptoms always had hepatomegaly and splenomegaly, and this was true for the majority of moderately severe cases. In the mild cases, however, the liver and spleen were seldom enlarged, although, tenderness on palpation in the right upper quadrant was often elicited. In the asymptomatic cases, the physical examination was negative. The allergic manifestations, namely, urticaria and angioneurotic edema, the eosinophilic reaction of the blood, and the symptoms related to the nervous and pulmonary systems were more frequent in the severe and moderately severe cases. The incidence of urticaria was, in percentages: 17.5 in the mild, 26.5 in the moderately severe, and 34 in the severe cases. Neurologic manifestations were not noted in the mild

cases but were present in 3 of the severe and 4 of the moderately severe cases. Spotty infiltration of the lungs, with increased peribronchial markings, was observed in one of the mild, one of the severe, and 6 of the moderately severe cases. As a rule, ova tended to be more numerous in the cases with the more severe symptoms. There were exceptions, however, as illustrated by absence of ova during the acute phase in several of the moderately severe cases as described above.

None of the cases in this series, to our knowledge, have been re-exposed to infested water. For this reason, the results of treatment are probably not comparable with those obtained in a civilian population, which is undergoing re-exposure year after year, and frequently day after day.

It was possible to make observations on 165 of the 210 cases treated with 40 cc. of fuadin from time to time over a period of from 4 to 28 weeks after completion of treatment. In a previous a paper (3) analysis of the data indicated that chemotherapy was followed by a rather uniform response. The symptoms and fever subsided within 2 to 3 weeks after the beginning of treatment, and this was observed irrespective of the duration of symptoms and fever prior to therapy. Coincidently, though less promptly, the enlarged and tender liver and the spleen receded. Subsequent clinical experience appears to substantiate these observations. Following the acute phase of the disease, all of the patients appeared to be well on physical examination. The temperature remained normal, the liver and spleen, if previously enlarged, returned to normal limits and tenderness disappeared. The majority, however, still complained of a varable degree of fatigue, anorexia, recurring mild upper abdominal discomfort and headache. In many cases, these symptoms disappeared within two to three weeks. In others, who were asymptomatic before treatment, mild symptoms developed following treatment. Some of the mild and asymptomatic cases remained on light or full duty during and after treatment, stating that they felt well and did not want to be hospitalized. In general, it was impossible to determine whether the post-treatment complaints were due to the disease, the effects of treatment or to psychogenic factors. The matter was evaluated by a psychiatric consultant (4), who pointed out that many of the complaints were undoubtedly due to emotional factors related to misapprehension concerning a disease

which was known to be new both to the patients and the physicians. The rapidity with which the epidemic struck brought together a large group of patients with the same disease, some of whom were seriously

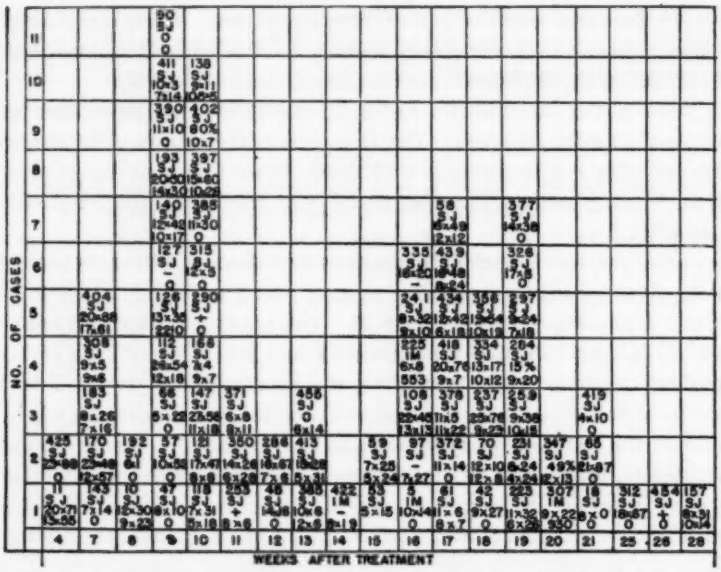


CHART 2. SCHISTOSOMIASIS JAPONICA—NON-RELAPSES—74 CASES TREATED WITH 40 CC. (0.340 GMS ANTIMONY) FUADIN

Each square represents one case. Reading from above downward is case number, result of pre-treatment stool examination, the pre-treatment white blood cell count (× 1,000) and percentage of eosinophiles (× 1,000), and the post-treatment blood examination. Week following treatment represents time of re-examination, and duration of post-treatment observation. Compare with Chart 1. S.J.—mature ova; I.M.—immature ova; —— indicates no data; 0—negative; %—eosinophilia. In cases 126, 225, and 307 total eosinophilic values are given.

ill. The relatively long period of hospitalization encouraged the spread of rumors regarding prognosis. An additional factor was the prevailing hope among the patients that their illness would lead to a return to the United States. In this setting, dissatisfaction, fears of the future, anxiety and resentment arose, and were associated with

mild, variable and vague complaints. In some of the patients, it was felt that the symptoms were genuine, but the symptoms characterizing the initial illness did not recur, except in 4 cases to be discussed below.

TABLE XI

Schistosomiasis japonica—relapse cases—Comparison of pre and post-treatment eosinophilia and leucocytosis correlated with severity of symptoms in 54 cases treated with 40 cc. fuadin

This table summarizes data presented in Chart 1, and is to be compared with Table XIII, which summarizes comparable data on Non-Relapse Group, presented in Chart 2. Post-treatment blood examinations made at time of relapse; post-treatment trend of eosinophilia based on these examinations compared with pre-treatment eosinophilia. It is to be noted that percentages for pre and post-treatment eosinophilia in Relapse Group is slightly higher, but are about equal for leucocytosis. There is, however, a marked difference in trend of post-treatment eosinophilia; in the Relapse Group, fewer cases show a decrease, more show an increase or do not change. (Percentages are based on actual number of cases examined.)

* Data on one case not adequate for analysis.

SEVERITY OF SYMPTOMS	NO. OF CASES	EOSINOPHILIA						LEUCOCYTOSIS						TREND OF EOSINOPHILIA		
		Before Rx			After Rx			Before Rx			After Rx					
		+	0	No data	+	0	No data	+	0	No data	+	0	No data	Down	Up	No change
Mod. severe	29	29	0	0	19	4	6	19	9	1	9	14	6	16	1	6
Mild	24	21	2	1	19	2	3	11	10	3	7	12	5	9	5	5
Asymptomatic	1	1	0	0	1	0	0	1	0	0	1	0	0	0	0	1
Total cases	54*	51	2	1	39	6	9	31	19	4	17	26	11	25	6	12
Percentage		96.3	3.7		71.4	28.6		59.6	40.4		37.7	62.3		60.0	13.3	26.7

* Data on one case not adequate for analysis.

(*b*) *Relapses following Treatment with 40 cc. of Fuadin:* To compare the data on the relapse and the non-relapse cases, reference should be made to Chart 1 and Table XI (relapses) and Chart 2[6] and Table XIII.

Studies on 165 cases, covering a period of from 4 to 28 weeks after treatment, are available. 55 (33 per cent) showed evidence of relapse or reactivity of the disease during this period. The diagnosis of relapse was based solely on the reappearance of ova in the stools in 50, and on the combination of symptoms, eosinophilia and ova in 3, and

[6] Because results of blood examinations were not available only 74 of the 110 non-relapse cases are represented in Chart 2, and of these the data on 64 are analyzed in Table XIII.

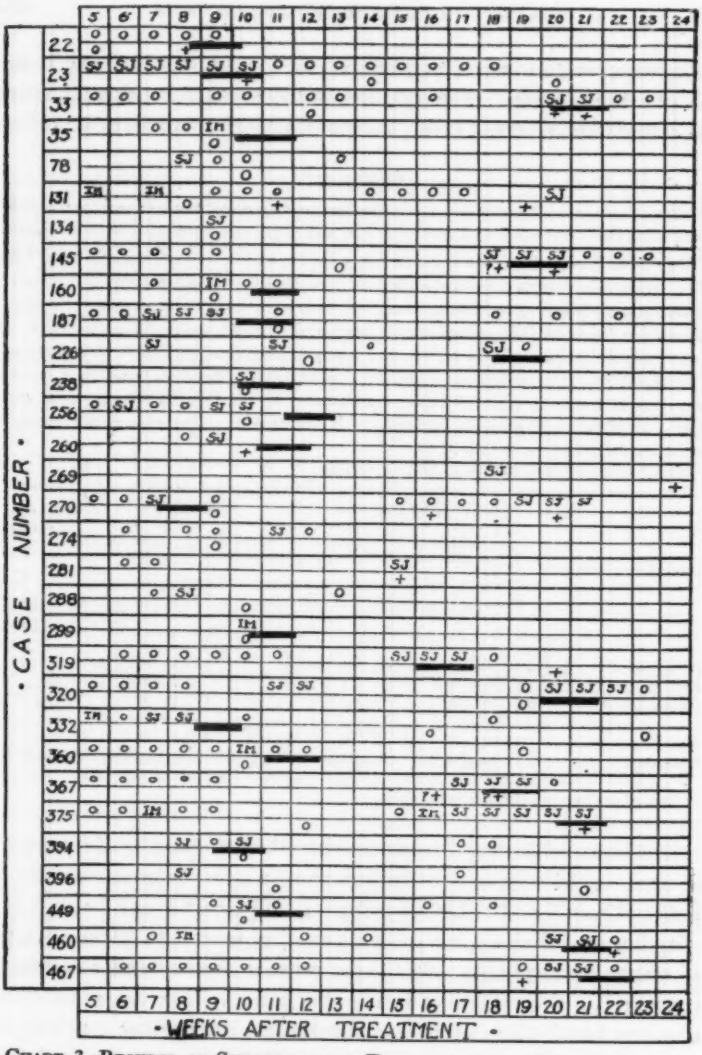


CHART 3. RESULTS OF SIGMOIDOSCOPIC EXAMINATIONS IN RELAPSE CASES

14 positive for intestinal nodules—17 negative. Upper line = ova of S. japonica. Lower line = intestinal nodules. S.J. = mature ova. I.M. = immature ova. ▬▬ = treatment.

symptoms, eosinophilia and intestinal nodules, without ova, in another. Only the latter 4 cases had objective clinical findings. Relapses were first detected in the 4th week after termination of treatment. The majority were detected during the 7th to the 10th weeks inclusive, in which period ova were found in 31 cases (57 per cent). Thereafter, the number of relapses decreased gradually through the 21st week.

Because symptoms accompanying relapse have been so infrequent, the 4 cases[7] in which symptoms were observed are of considerable interest. In Case 22 mature ova had been found before treatment was started. The presenting symptoms were mild, the leucocyte count was 9,000 of which 31 per cent were eosinophiles. Symptoms improved after treatment, but in the 7th week they recurred along with slight afternoon fever. In the 8th week, the white blood cells were 14,000 with 58 per cent eosinophiles; the edge of the liver was palpable and slightly tender; intestinal nodules were found on sigmoidoscopic examination, but ova were not found in the stools (Chart 3).

Case 159 exhibited mild symptoms of schistosomiasis during the recovery phase of acute hepatitis, which was accompanied by pulmonary infiltration of the left lower lobe. Mature ova were present in the stools. The white blood cells and eosinophiles were not increased. Improvement clinically and in the pneumonitis followed chemotherapy. In the fourth post-treatment week, slight fever and signs of pneumonitis in the same area recurred; an eosinophilia of the blood had now developed. Only immature ova were found in the stools. With retreatment symptoms and signs again receded. In Case 360, slight fever recurred in the 10th post-treatment week; the eosinophilia and leucocytosis, both high on admission, remained elevated; only immature ova were reported in the stools; the sigmoidoscopic examination was negative. Seven weeks after retreatment he was apparently well, the stools were negative, and white blood cells and differential were normal. Case 457 exhibited slight fever, upper abdominal discomfort, slightly enlarged and tender liver, palpable spleen and increased eosinophilia in the 9th post-treatment week. Again only immature ova were found in the stools. Improvement followed retreat-

[7] Since the data in this study were analyzed and assembled, a fifth case, No. 131 (discussed in Sect. IV), has relapsed and definite symptoms developed. This makes a total of 56 relapses.

TABLE XII

Schistosomiasis Japonica—3 Cases Each Showing Two Relapses

Table illustrates that the effect of Antimony in causing ova to disappear in stools is only temporary (at least in these cases); that the interval between relapses is fairly uniform, despite differences in amount of Antimony given; and that severity of symptoms accompanying initial illness is unrelated to persistence of disease. Except in Case 270, leucocytes and eosinophilia decreased and did not rise during relapse. Sigmoidoscopic examination positive for intestinal nodules in Case 270. SJ—Mature ova;—No data available; **—Indicates period of treatment.

CASE NO.	SEVERITY OF SYMPTOMS		WEEKS OF DISEASE																						
			Initial illness								First relapse									Second relapse					
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
325	Mod. severe	Stool exam.	SJ				0	0	0				0	SJ							SJ	SJ	SJ	SJ	0	0		
		Fuadin	********** 40 cc.											******** 70 cc.									******* 60 cc.					
		WBC × 1,000	—											—								4.1					
		E-% × 1,000	43%											—								8					
		Sigmoidoscope ex.																					0					
364	Mild	Stool exam.	SJ				0		0	0	SJ	SJ	0	0							0	SJ	SJ	SJ	SJ	0		
		Fuadin		********** 40 cc.							**** 40 cc.												******* 60 cc.					
		WBC × 1,000	10							5.5												6.2					
		E-% × 1,000	15							12												8					
		Sigmoidoscope ex.																						0				

| | | |

ment with 60 cc. fuadin, and 5 weeks after completion of treatment stools were negative and the eosinophilia, though markedly reduced, still persisted.

Reference should now be made to Tables XI and XIII which summarize the incidence of the pre- and post-treatment leucocytosis, the percentages of eosinophiles and the trend of the post-treatment eosinophilia in the relapse and non-relapse cases, respectively. These findings are correlated with the severity of symptoms noted in the initial illness. It is to be noted that the percentages for pre- and post-treatment eosinophilia in the relapse group is higher than in the non-relapse group, but the figures for leucocytosis are about equal in the two. Here it should be stated that the eosinophilia during the initial illness and while under treatment, as reported in a previous study (3), fluctuated considerably in the individual patient, but no uniform trend was found. In the post-treatment period the same variations occurred, but in general, the trend was downward in both relapse and non-relapse cases during the period of our observations. This downward trend was, however, much less evident in the relapse group, in which also an upward trend at the time of relapse occurred not infrequently. Table XIV illustrates that the incidence of hookworm, ascaris and other parasitic ova found in the stools were about the same in the relapse and non-relapse groups.

There is some correlation between the severity of symptoms and the relapse rate. Of the 165 cases observed, 55 had moderately severe symptoms in their initial illness, of which 29 relapsed (53.7 per cent); of the 104 mild cases, only 25 relapsed (23.8 per cent); and of the 6 asymptomatic cases, 1 relapsed (16.3 per cent). Only 1 severe case was followed for more than 4 weeks during the post-treatment period. Ova did not reappear in this case during 11 weeks of observation.

(*c*) ***Multiple Relapses in Cases originally treated with 40 cc. Fuadin:*** Three cases have been observed through two relapses.[8] The data are summarized in Table XII. Again it may be seen that the effect of chemotherapy upon the ova in the stools was only temporary. The Table shows that the interval between relapses though variable, was

[8] Since data in this study were assembled and analyzed, a 4th case has relapsed a second time.

TABLE XIII

Schistosomiasis japonica—Non-Relapse Cases—Comparison of Pre and Post-Treatment Eosinophilia and Leucocytosis Correlated with Severity of Symptoms in 65 Cases Treated with 40 cc. Fuadin

This table summarizes the data presented in Chart II, and is to be compared with Table XI which summarizes the data on the Relapse Cases. Percentages for pre and post-treatment eosinophilia in the Non-Relapse Cases are slightly lower, but are about the same in both groups for leucocytosis. The downward trend of the post-treatment eosinophilia is, however, more marked in the Non-Relapse Cases, and fewer cases show an upward trend.

SEVERITY OF SYMPTOMS	NO. OF CASES	EOSINOPHILIA				LEUCOCYTOSIS				TREND OF EOSINOPHILIA		
		Before Rx		After Rx		Before Rx		After Rx		Down	Up	No change
		+	0	+	0	+	0	+	0			
Severe	1	1	0	1	0	1	0	0	1	1	0	0
Mod. severe	16	12	4	9	7	9	7	2	14	14	2	0
Mild	43	39	4	28	15	28	15	8	35	37	0	6
Asymptomatic	5	3	2	2	3	2	3	1	4	2	0	3
Total cases	65*	55	10	40	25	40	25	11	54	54	2	9
Percentage		84.6	15.4	61.5	38.5	61.5	38.5	17	83	83	2.9	14.1

* Available data on 45 non-relapse cases inadequate for analysis.

TABLE XIV

Schistosomiasis japonica—Comparison of Incidence of Parasitic Ova other than S. Japonicum in Relapse and Non-Relapse Cases

There is essentially no difference in incidence between the two groups, so that any influence on eosinophilia could be expected to be about the same. Cases in Non-Relapse Group were observed for same period of time as in Relapse Group.

SEVERITY OF SYMPTOMS	TOTAL CASES	RELAPSES—54 CASES					TOTAL CASES	NON-RELAPSES—59 CASES				
		H.W.	S.T.	T.T.	E.H.	A.L.		H.W.	S.T.	T.T.	E.H.	A.L.
Mod. severe	29	6	1	2	3	2	26	5	0	4	2	4
Mild asymptomatic	24	8	2	1	2	2	28	8	0	0	2	2
Asymptomatic	1	0	0	0	0	0	5	1	0	0	0	0
Totals	54	14	3	3	5	4	59	14	0	4	4	6

Abbreviations:

H.W.—Hookworm ova.
S.T.—Strongyloides ova.
T.T.—Trichuris ova.
E.H.—Endamoeba histolytica.
A.L.—Ascaris ova.

fairly uniform; and further, that the variations in this interval were not related to the amount of, nor to the rate of administration of the drug. The interval between the first and second relapse was 5, 9 and 10 weeks, respectively, following 70 cc. in 22 days in Case 325, 40 cc. in 8 days in case 364, and 60 cc. in 12 days in case 270. In the second relapse as in the first, symptoms did not develop although the loss of weight accompanying the initial illness was not regained by cases 364 and 270. The trend of the eosinophilia was downward in each of these cases. The leucopenia observed in these cases has not been observed in

TABLE XV

Sigmoidoscopic examinations—comparison of relapse and non-relapse group

14 of the 31 relapse cases examined showed intestinal nodules, compared to only 7 of the 80 Non-Relapse cases. Note that majority of nodules were found after the 17th Post-Treatment week.

CASES	RESULT OF SIGMOID EXAM.	WEEKS AFTER TREATMENT																						
		6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	Total
31 Relapse	Positive			1		2					1			1	2	4	1	1		1				14
	Negative				4	8	1	1							1		1		1					17
80 Non-relapse	Positive					1		1			2					2							1	7
	Negative	4	3	7	13	14	5	6	4	2	1	3	2	2	5	1							1	73
Totals		4	3	8	17	25	6	8	4	2	4	3	2	3	8	7	2	1	1	1			2	111

other cases. It may be related to the chemotherapy, since it was not observed in the non-relapse cases treated with only one course of fuadin (40 cc.).

IX. SIGMOIDOSCOPIC EXAMINATIONS IN CASES TREATED WITH 40 CC. OF FUADIN

113 cases were examined from 2 to 26 weeks after completion of their initial treatment. Lesions such as those described by Johnson and Berry (2) were found in some of the cases. The lesions were of three types. The first consisted of solitary yelllowish nodules from 1 to 2 mm. in diameter, situated in the submucosa. There was no evidence of any surrounding inflammatory reaction. The second, more frequent type was a grouping of from 5 to 10 small nodules, much like the above, but in this type, the nodules were surrounded by a zone of induration forming a plaque-like area in the submucosa. These plaques were

often as large as 1.5 cm. in diameter, although they were not always circular in form. The third type was the least common; it resembled the second type, except that there was definite superficial ulceration of the lesion. The commonest site for all of the lesions described was from 10 to 15 cm. above the pectinate line, although, some were seen above and some below on the Hausterian valves in the rectum.

In 4 cases, biopsies of the nodules were made. In cases 33 and 460, which were undergoing treatment, both immature and degenerate ova were found. In the other two cases, 319 and 401, in which examinations were made during the 3rd and 6th post-treatment week, only degenerate ova were found.

Examinations were made on 31 cases in relapse (Chart 3). 14 showed typical lesions in the submucosa, and 17 were negative. 22 of the examinations, 11 of which were positive and 11 negative, were made within one week's time of the positive stool examination.

In 80 cases, in which the stools never became positive after treatment, 7 were found to have intestinal nodules on sigmoidoscopic examination. In this group the examinations were made on the average earlier in the post-treatment period than in the relapse group, which may be of some significance. It is possible that lesions developed in additional cases in later weeks.

Special attention is called to case 22 (Chart 3). Ova were not found in the stool on repeated examinations, and the diagnosis of relapse was based on the symptoms and the nodules in the bowel. The other 7 cases in which nodules were found in the absence of positive stool examinations, were not treated because they were asymptomatic and the significance of the nodules could not be evaluated. In 2 cases, 131 and 270, intestinal nodules were found before ova were detected in the stools, indicating the probable diagnosistic value of the sigmoidoscopic examination. In view of the accumulated experience, it is now believed that treatment should be repeated if nodules not previously noted should appear in the bowel more than 6 weeks after the completion of the previous course of therapy, even in the absence of symptoms and of ova in the stools.

DISCUSSION

The epidemic of schistosomiasis japonica which has been described, involved a previously unexposed population, and closely resembled in

its general characteristics, explosive outbreaks of acute infectious disease caused by bacteria or filterable viruses. It developed slowly, then accelerated rapidly and subsided gradually. The clinical manifestations, severe in the early phase, became milder as the epidemic progressed, and finally, the asymptomatic or sub-clinical form of the disease was uncovered. Furthermore, a state of immunity appeared to develop, for once the acute symptoms of the initial illness subsided, they recurred in mild form in only 4 cases, in spite of the continued presence of the infecting agent. These similarities could only exist when large numbers of previously unexposed troops were exposed to the schistosome within a relatively short period of time, a situation usually existing in epidemics caused by other agents. As in other epidemics, a certain proportion of the exposed individuals did not contact schistosomiasis (3). This may be dependent upon the balance between the size of the infecting dose and host resistance.

Although the disease is more easily recognized in its more severe form, the mild type of the disease has been more frequently encountered. The disability complained of by patients with mild symptoms may be relatively slight. Consequently, the diagnosis may be delayed in some instance for weeks and even months after the onset of symptoms. The importance of recognizing the asyptomatic or subclinical form of the disease cannot be overemphasized. The absence of eosinophilia in some of the asymptomatic and, also, in some of the symptomatic cases as well, may be misleading. To uncover the asymptomatic or sub-clinical cases, surveys must include examinations of the stools as well as the blood. In many cases, repeated examinations of the stools over a period of weeks and months may be necessary to find ova. In a few cases only immature ova may be found during the early weeks of the disease.

Observations made during chemotherapy throw some light on the mode of action of antimony. Both mature and immature ova disappear from the stools during the course of therapy, but their viability, as far as can be determined, is not affected by the drug. The disappearance of ova is presumably due to cessation of ovulation. That the inhibition of ovulation is only temporary is well illustrated by the reappearance of ova in more than one-third of the cases; and in a few cases, after a second course of treatment.

Since the ova are probably the chief cause of the clinical and pathological changes in schistosomiasis, the subsidence of symptoms coincident with cessation of ovulation seems logical. The absence of symptoms in the great majority of patients at the time of reappearance of ova in the stools is of particular interest. Symptoms of a mild nature were noted in only 4 cases. It would appear that following the acute phase, a state of immunity develops, and concurrently, the allergic component of the host reaction subsides. With resumption of ovulation symptoms due to allergic hypersensitivity seldom reappear.

The relapse rate following treatment with 40 cc. of fuadin was approximately 30 per cent of the 165 patients on which we have information:[9] It is obvious, however, that without a control series of untreated cases no conclusions can be reached concerning the effect of this form of treatment upon the ultimate course of the disease.

SUMMARY

1. Observations are reported on 364 cases of acute schistosomiasis japonica admitted to an Army General Hospital on the island of Leyte, P. I., in the winter of 1944–45.

2. The first patients developed symptoms in November, one month after the landing of United States troops on the island. The outbreak reached its peak in January, then gradually subsided, and came to an end in May.

3. In general, the more severe cases were seen early in the outbreak. Mild and subclinical forms of the disease became increasingly evident while the outbreak was subsiding.

4. The importance of recognizing the existance of the asymptomatic form of the disease is emphasized.

5. During an outbreak in an endemic area, a small percentage of cases (7 cases in the present series), having characteristic clinical and laboratory manifestations of schistosomiasis, may not have ova in their stools.

[9] To this should be added 15 additional relapses in cases in this series treated with 40 cc. of fuadin according to information kindly forwarded by Lt. Col. Worth Daniels based on studies on patients returned to the United States after completion of treatment. This gives a total of 70 relapses (42 per cent) in 165 patients on whom we have information.

6. In the pre-treatment stool examinations, mature ova were found in 344 cases. Only immature ova were found in 16 cases; in which after treatment had been instituted mature ova were found.

7. All patients were treated with an antimony compound: fuadin, tartar emetic or anthiomaline. Only the results obtained in 210 patients treated with a total dose of 40 cc. of fuadin, are reported in detail at this time, but it may be said that relapses have occurred after all forms of treatment.

8. Only mild toxic effects in 2 patients were noted during the treatment with fuadin.

9. During treatment with fuadin, ova disappear from the stools usually between the 10th and 16th days of treatment. The viability of the eggs appears to be unaffected by fuadin, and their disappearance from the stools is probably attributable to the effect of the drug upon ovulation in the female worm.

10. The symptoms of acute schistosomiasis subside at approximately the same time that the eggs disappear from the stools.

11. Relapses following one course of 40 cc. of fuadin have been encountered in approximately 40 per cent of the cases observed for more than 4 weeks after termination of treatment. In only 4 of 55 relapses were symptoms of schistosomiasis noted. In all other cases the relapse was characterized solely by the reappearance of ova in the stools. More than half of the relapses were noted between the 7th and 10th week following termination of the course of treatment. Relapses have also been observed after a second course of treatment.

12. The tendency to relapse appears to be related to the severity of the initial illness. Relapses are more likely to occur in those who have a persistent eosinophilia following the first course of treatment.

13. The finding of characteristic intestinal nodules, on sigmoidoscopic examination, may be helpful in detecting a relapse; they occur in the absence of ova in the stools.

14. Larger doses of fuadin, up to a total of 80 cc. in a single course, have been administered without encountering any serious toxic effects. The therapeutic effect of these larger doses cannot be analyzed until a later date.

Note: The authors would like to express their appreciation to the Chiefs of Medicine of the several General Hospitals on Leyte for supplying the post-treatment

data on many of the cases in this study. Many of the stool examinations were made by Capt. O. H. Graham, Sn.C., and Capt. M. S. Ferguson, Sn.C. of the United States Army 5th Malaria Survey Unit and grateful acknowledgment is extended to them for the inclusion of their results in this study. Further, appreciation is due Capt. F. B. Bang and 1st Lt. N. G. Hairston for helpful information of an experimental nature and for permission to refer to their experiments now in progress. Lastly, many of the data were assembled and tabulated by Pfc. Walter Farr and T/5 Ralph Goldenberg.

BIBLIOGRAPHY

1. Thomas, H. M. Jr., and Gage, D. P.: Symptomotology of Early Schistosomiasis japonica. Bull. U. S. Army Med. Dept. Vol., IV. No. 2, pp. 197, Aug.,'45.
2. Johnson, A. S., Jr., and Berry, M. C.: Asiatic Schistosomiasis: Clinical Features, Sigmoidoscopic Picture and Treatment of Early Cases. War Medicine, **8**, 156, Sept. 1945.
3. Billings, F. T., Winkenwerder, W. L., and Hunninen, A. V.: Studies on Schistosomiasis japonica in the Philippine Islands. 1. A Clinical Study of 337 Cases with a Preliminary Report on the Results of Treatment with Fuadin in 110 Cases. Bull. J.H.H. **78**, 21–56, Jan. 1946.
4. Frank, J. D.: Emotional Reactions of American soldiers to an Unfamiliar Disease. Amer. Jr. Psychiatry. **102**, 631–40, Mar. 1946.
5. Sullivan, R. R., and Ferguson, M. S.: An Epidemiological Study of Schistosomiasis japonica.
6. Carroll, D. G.: Cerebral Involvement in Schistosomiasis japonica. Bull. J.H.H. **78**, 219–234, Apr. 1946.
7. Vogel, Hans.: Ueber Entwicklung, Lebensdauer, und Tod der Eier von Bilharzia im Wirtsgewebe. Duet. Trop. Ztschr. **46**, No. 3 and 4, pp. 57–69; 81–91, 1 and 15 Feb., 1942.
8. Fairley, N. H.: Quoted by Strong in Stitt's *Diagonosis, Prevention and Treatment of Tropical Diseases.* Vol. II, pp. 1416. The Blakiston Co., 1942, Philadelphia.
9. Bang, F. B., and Hairston, N. G.: Personal Communication.

THE PROBLEM OF IDENTIFICATION OF ORAL SPIROCHETES AND DESCRIPTION OF A PRECIPITIN TEST FOR THEIR SEROLOGICAL DIFFERENTIATION

LUCILLE B. ROBINSON, A.B. AND RUTH H. WICHELHAUSEN, M.D.*

From the Department of Bacteriology, Johns Hopkins University School of Medicine, Baltimore, Md.

The classification and identification of spirochetes found in the oral cavity, throat and respiratory tract have been based largely on their morphological characteristics. Confusion has arisen not only because a very large number of different spirochetes has been described but also because in many cases there is little agreement between various authors as to the proper naming or classification of the organisms they have studied.

The controversies that have arisen with the subsequent loss of much valuable work because of the questionable identity of the organisms involved may be illustrated by the following chronological list of spiral organisms.

1. *Treponema buccale.* The organism was extensively studied by Dobell in 1912 (17) and the name of *T. buccale* was proposed in preference to the commonly used name *Spirochaeta buccalis* Cohn 1875 (12). According to Fantham (19) it should be named *Spirochaeta buccalis* Steinberg 1862. In the opinion of Smith and others (16, 22, 51, 60) it may be identical with *Spirochaeta bronchialis* Castellani 1907, also with *Sp. inaequalis* Gerber 1910 and with *Sp. undulata* Gerber 1910. Hoffmann in 1920 indicated that there might be three forms of *Sp. buccalis* Cohn: *Sp. buccalis crassa*, *Sp. buccalis tenuis* and *Sp. buccalis inaequalis* (25).
2. *Treponema dentium.* The name was suggested by Dobell in 1912 (17). The organism was first described by Koch in 1877 as "Spirochaete des Zahnschleims" (32). It was named *Spirochaeta dentium* or *Spirochaeta denticola* by Miller 1889 (40). Veillon in 1894 (62) described a *Treponema dentium* and *T. denticola* which may be the identical organisms. Gerber's *Sp. dentium* and *Sp. denticola* may be identical according to the author himself 1910 (22). Noguchi (42) includes *T. microdentium* and *T. macrodentium* under the general specific name *dentium* in contrast to the "buccalis" type. Hoffmann in 1920 (25) describes *Spirochaeta orthodonta*, *Sp. skoliodonta* and *Sp. trimerodonta* as

* This work was aided by funds received from an anonymous donor through Dr. Louis D. Coriell.

variations ("Abarten") of *T. dentium* Koch 1877, *Sp. orthodonta* being closest to the classical organism.

3. *Borrelia refringens* Schaudinn 1905 has been reported as an inhabitant of the oral cavity by various authors (22, 46, 73), even though it is not generally considered an "oral" spirochete.
4. *Borrelia vincenti* R. Blanchard 1906 (3, 5, 49, 63, 64). Synonyms according to Bergey (1939) are: *Spiroschaudinnia schaudinni* Prowazek 1907, *Spiroschaudinnia vincenti*, *Treponema vincenti*, *Spironema vincenti*. Blanchard called the organism *Spirochaete vincenti* (5) but Fantham (19) points out that the species name is probably not valid as no accurate description of the spirochete was given at the time of the naming. *B. vincenti* may be identical with *Sp. tenuis* Gerber and *Sp. recta* Gerber (22, 60), with *T. schaudinni* (46), with *Sp. bronchialis* Castellani (61, 67), with *Sp. minima* Beaurepaire-Aragao et Vienna 1913 (7, 48) or with *Sp. nosocomicalis* Hoffmann (7).
5. *Spirochaeta bronchialis* Castellani 1907 (19). Synonym: *Spiroschaudinnia bronchialis* Castellani 1907 (3). Fantham (19) and Besançon and Etchegoin (4) consider the organism a distinct species, whereas Smith and others (16, 51, 60) believe it to be the same organism as *T. buccale*. According to Strong (61) and Vincent (67) it is identical with *B. vincenti*.
6. *Spiroschaudinnia subtilis* Castellani 1907 (3). It may be identical with *T. dentium* (7, 48). See also No. 2.
7. *Spirochaeta gracilis* Veszpremi 1907 is probably the same as *T. dentium* (48). See also No. 2.
8. *Spiroschaudinnia aboriginalis* Cleland 1909 (3, 9).
9. *Spirochaeta inaequalis* Gerber 1910 (22) may be identical with *Sp. undulata* Gerber 1910 (22) and/or with *T. buccale* (22, 60). See also Nos. 1 and 10.
10. *Spirochaeta undulata* Gerber 1910 (22) may be identical with *Sp. inaequalis* (22) and/or with *T. buccale* (22, 60). See also Nos. 1 and 9.
11. *Spirochaeta dentium* Gerber 1910 (22) may be identical with *T. dentium* (32), with *Spirochaeta denticola* Gerber (22), with *T. microdentium* (42, 60) or with *T. mucosum* (60). See also Nos. 2, 12, 16, 17.
12. *Spirochaeta denticola* Gerber 1910 (22) may be identical with *Sp. dentium* Gerber. See No. 11.
13. *Spirochaeta tenuis* Gerber 1910 (22) is considered a possible variety of *B. vincenti* or *T. buccale* (22), the same organism as *B. vincenti* (60), the identical organism as *T. macrodentium* (57), the same organism as *Spirocheta media oris* (25, 48) or *T. intermedium* (17). See also Nos. 1, 4, 15, 18.
14. *Spirochaeta recta* Gerber 1910 (22) is considered a possible variety of *T. buccale* or *B. vincenti* (22) or as identical with *T. macrodentium* (60). See also Nos. 1, 4, 18.
15. *Treponema intermedium* Dobell 1912 (17) is according to Dobell the same as the "mittlere Spirochaetenform" of Hoffmann and Prowazek 1906 (24), later referred to by Hoffmann as *Sp. media oris* (25).

16. *Treponema microdentium* Noguchi 1912 (42) may be identical with *Sp. dentium* Gerber or *Sp. denticola* Gerber (22, 60), with *T. intermedium* (17) or with *T. mucosum* (68). See also Nos. 2, 11, 12, 15, 17.
17. *Treponema mucosum* Noguchi 1912 (43) may be identical with *T. microdentium* (68, 69). See Nos. 2 and 16.
18. *Treponema macrodentium* Noguchi 1912 (42). According to Vinzent and Daufresne *Sp. tenuis* Gerber is a variety of (68) or identical with (57) *T. macrodentium*. According to Smith (60) *Sp. recta* Gerber may be identical with *T. macrodentium*, whereas Gerber himself considers both, *Sp. tenuis* and *Sp. recta* possible varieties of *T. buccale* or *B. vincenti* (22). Pettit mentions *Sp. macrodentium* as synonym of *Sp. buccalis* fide Brumpt 1922 (7, 48). See Nos. 1, 4, 13, 14.
19. *Form A and B of Sangiorgi* (56) probably correspond to *T. buccale* and *T. dentium* respectively (48). See Nos. 1 and 2.
20. *Spiroschaudinnia minuta* Castellani 1916 (3), identical with *Spirochaeta minuta* (10).
21. *Spirochaeta buccopharyngei* Macfie 1916 (48) is probably the same as *Sp. dentium* (7). See No. 2.
22. *Treponema orthodontum* Hoffmann 1920, described by Hoffmann (25) as *Spirochaeta orthodonta*, a variety ("Abart") of *Sp. dentium* Koch. See No. 2.
23. *Treponema skoliodontum* Hoffmann 1920, described by Hoffmann as *Spirochaeta skoliodonta* (25), a variety of *Sp. dentium* Koch, is believed identical with *Sp. acuta*. (37, 57, 68). See also Nos. 2 and 25.
24. *Leptospira dentium* Hoffmann 1920 (26), originally described as *Spirochaeta trimerodonta* (25), was considered the same organism as *Leptospira buccalis* by Vinzent and Daufresne (68), but following later studies (57, 70) it was decided that the two organisms were not identical. According to Pettit and Muttermilch "*Leptospira trimerodonta*" is identical with *L. buccalis* Fontana (41, 48).
25. *Spirochaeta acuta* Kritchewsky and Séguin 1920 (37) is probably identical with *T. skoliodontum* (57, 68). See No. 23.
26. *Spirochaeta perforans* Cavalié and Mandoul 1921 (7, 11, 48).
27. *Leptospira buccalis* Fontana (41) may be the same as *L. dentium*. See No. 24.
28. *Spirochaeta commandoni* Séguin and Vinzent 1936 (57, 69, 70).
29. *Spirochaeta ambigua* Séguin and Vinzent 1936 (57, 69, 70).
30. *Spirochaeta orogyrata* Séguin and Vinzent (69).

Many authors agree that the usual morphological description of spirochetes presents factors which are unreliable for the differentiation of spiral organisms. Since all these factors are dependent on environmental conditions, they are subject to more or less variation.

Much attention has been paid to the type of motion of spiral organisms, not only for the identification of oral spirochetes, but also to differentiate *T. pallidum* from the small oral spirochetes and the saprophytic spirochetes of the genital tract. The observation of the type of motion is a very valuable supplement for diagnosis

in the hands of experienced workers, but it should not be used to identify a new species. We quote Smith (60): "The variations due to the type of motion should not be used to identify a new species as many authors have attempted to do, since all these variations can be observed in the same individual."

Consequently several authors have begun to differentiate the various strains of spirochetes on the basis of their cultural and biochemical characteristics and their serological specificity rather than to depend on their morphology alone.

Several of the organisms mentioned above have never been grown on artificial media, whereas others have been obtained in culture only in rare instances. However in recent years several papers have appeared in the literature which indicate that at least some oral spirochetes may be cultivated with comparative ease, so that the routine isolation of these spirochete strains in a specialized laboratory is now possible (20, 23, 29, 50, 53, 54, 55, 58, 59, 69, 70, 72).

The strains obtained in culture have been identified largely on the basis of their morphological characteristics, but several attempts have been made to classify them also by their cultural characteristics and biological properties. The characteristics include ease of primary isolation and cultivation, dependence on essential growth factors, appearance of colonies, odor of culture, production of mucin, proteolytic and saccharolytic activity, hydrogen sulfide production, lysis in saponin and bile salts, and determination of oxidation-reduction potential in cultures (1, 15, 42, 43, 50, 54, 57, 69, 72).

The information procured by these means has marked a definite progress in the identification of strains studied in various laboratories. However, these data are still quite incomplete. This may be due to two factors.

1. Different workers have used different culture media and different biological tests so that it is difficult to compare their results. The only identifying features that are mentioned with any regularity are: a) the essentiality or non-essentiality of fresh tissue, b) the production of mucin, c) the presence or absence of a fetid odor of the cultures. Even if only these three differentiating points are used, there is not always agreement among the authors (42, 57).
2. Evidently the oral spirochetes have a limited power to attack various substances, so that for instance the testing of the saccharolytic activity of the various organisms, so widely used for the identification of bacteria, seems to offer little or no advantage. However it has been possible to demonstrate proteolytic activity in various strains and to use it in the differentiation of oral spirochetes.

Spirochetal antigens, prepared from strains of oral spirochetes have been used to identify and classify the oral spirochetes as well as to evaluate the relation of syphilitic reagin to spirochetal antibody. Kolmer (36) gives a good review of the latter studies, in which in many cases oral spirochetes in conjunction with *pallidum* strains were employed. Precipitin tests were done occasionally, but agglutination and complement fixation tests were the methods of choice. In the work concerning the oral spirochetes specifically the tests employed were almost exclusively agglutination tests.

It has been reported that non-syphilitic human sera have high agglutinin and complement fixing titers against *T. microdentium*, *T. macrodentium*, *T. dentium* and various *pallida* strains (2, 34, 35, 36). Normal rabbit serum agglutinated *T. macrodentium* (dil. 1:50) and *T. microdentium* (dil. 1:200) (36).

Absorption of syphilitic serum with the Reiter strain removed agglutinins for the Reiter strain, but not for *T. macrodentium* and *T. microdentium* (35).

Complement fixation and agglutination tests have demonstrated cross reactions between oral spirochetes (*T. macrodentium* and *T. microdentium*) and the Nichols-Hough, Noguchi, Kroo and Zinsser A strains of *T. pallidum* (35, 36).

Noguchi (45, 50) found that *T. macrodentium* is agglutinated in *T. macrodentium* antiserum, but gives no cross reactions with *T. microdentium* and *T. mucosum*, whereas *T. microdentium* and *T. mucosum* cross react, but do not react with *T. macrodentium*.

However Vinzent and Seguin state that there is never any cross reaction between strains belonging to different species (57). In agglutination tests performed later (69) they found that antiserum against one strain of *T. microdentium* failed to agglutinate *Sp. ambigua*, *Sp. commandoni*, *Sp. macrodentium* and *Sp. calligyrum* and agglutinated only seven out of eight strains of *T. microdentium*. This suggested to the authors the possible existence of a sub-type of *T. microdentium*.

Differentiation of oral spirochetes by means of agglutination tests has not been satisfactory in our hands. Agglutinating sera with a titer of 1:2048 were obtained, but there were cross-reactions between morphologically definitely distinct strains in serum dilutions to 1:256. We also found a number of normal rabbit sera that agglutinated spirochetal suspensions in dilutions to 1:128, which agrees with the findings of Kolmer (36). It would probably be possible to overcome these difficulties by specific absorption of the sera or by the use of high serum dilutions. However, we have not carried this phase of our study very far, partly because of the positive reactions obtained in normal sera and mostly because we became interested in a precipitin test as a possible means of differentiating oral spirochetes.

We wish to report on a precipitin test by which we have been able to differentiate oral spirochetes and also a few alleged *pallidum* strains, some of which were morphologically or culturally distinct, others morphologically and culturally indistinguishable from each other.

The strains of spirochetes used in this study included 19 strains of oral spirochetes, isolated from cases of Vincent's infection and pyorrhea alveolaris and 4 strains of alleged *T. pallidum*, the Reiter, Kasan, Nichols and Noguchi strains. These spirochetes could be divided into

4 groups according to their morphology and cultural characteristics as illustrated briefly in Table I.

CULTIVATION OF SPIROCHETES FOR THE PREPARATION OF ANTIGEN

The spirochetes used for the immunization of rabbits and for the preparation of spirochetal extracts were grown in 500 cc. Florence flasks. The medium consisted of a pancreatic digest broth[1] containing 2-mercapto-ethanol[2] as a reducing agent in a concentration of 1:1000 and methylene blue in a concentration of 1:1,000,000.

Mercapto-ethanol has proved a satisfactory reducing agent for our purposes. It was found non-toxic to any of the spirochetes studied in concentrations of 1:500 and 1:1000. Anaerobiosis was obtained quickly with a concentration of 1:1000 mercapto-ethanol, and it was maintained in this liquid medium for 4 weeks or longer. The medium will become aerobic sooner if a large surface of it is exposed. If Florence flasks were filled with medium to about one to three cm. above the constriction of the flask, only the upper one or two cm. became aerobic eventually.

To the above medium was added 10% of ascitic fluid or 1% of defibrinated rabbit blood, depending on the strain to be cultivated (see Table I).

The flasks were incubated at 37°C for 14 to 24 days and the contents were distributed into pointed centrifuge tubes and centrifuged at 3,600 to 4,000 R.P.M. for one hour. The supernatant fluid was discarded, the sediments combined, washed with 0.85% NaCl solution several times and then used either for the immunization of rabbits or for the preparation of spirochetal extracts.

The sediment of the cultures grown with the addition of rabbit blood contained besides the spirochetes a number of red cells and/or cell debris. With an incubation time of 2 to 3 weeks most of the blood became hemolyzed, so that few, if any, intact blood cells were left. If the sediment contains too many blood cells, it is possible to eliminate them by fractional centrifugation. Centrifugation at lower speed (1,600 R.P.M.) will throw the blood cells into the tip of the centrifuge tube whereas the lighter spirochetes will remain in the upper layer and may thus be separated.

It seemed preferable to work with cell-free media and to substitute hemoglobin solutions for blood, but attempts to do so have failed for two reasons.

1. The growth of spirochetes is more satisfactory in media containing defibrinated blood than in those containing hemoglobin.

2. Addition of hemoglobin solutions to mercapto-ethanol broth resulted in the formation of a heavy sediment.

---

[1] Pancreatic digest broth: To 1000 gms. of finely ground beef heart were added 4000 cc. of dist. water. After heating to 56°C. 10 gms. of pancreatin (Balt. Biol. Lab.) and 20 gms. of NaCl were added, the mixture kept at 56°C. for 2 hours, boiled for 5 minutes, filtered and adjusted to a pH of 7.4.

[2] From Carbide and Carbon Chemicals Corporation.

TABLE I

CLINICAL DIAGNOSIS	PATIENT		MORPHOLOGY	GROWTH IN BLOOD AGAR	BLOOD NECESSARY FOR STOCK MEDIUM	ASC. FL. OR SERUM SUFFICIENT FOR STOCK MEDIUM	ANTIGEN	ANTISERA A 1	A 2	A 3	A 4	B 1	B 2	C 1	D 1	D 2	E	F	C 2	G	H	T	K 1	C 3	L	K 2	Reiter	Kasan	Nichols	Noguchi	Normal serum	NaCl	Mercapt. broth and blood	SEROLOGICAL GROUP
Vincent's infection	A	A-1	Small regularly coiled spirochetes	+ hemolytic	+	−	A-1	+	+	+	+	+	+	+																			
		A-2					A-2	+	+	+	+	+	+	+																			
		A-3					A-3	+	+	+	+	+	+	+																			
		A-4					A-4	+	+	+	+	+	+	+																			
Acute ulcero-membranous gingivitis	B	B-1					B-1	+	+	+	+	+	+	+																			1
		B-2					B-2	+	+	+	+	+	+	+																			
Vincent's infection	C	C-1					C-1	+	+	+	+	+	+	+																			
Vincent's infection	D	D-1					D-1								+	+	+															
		D-2					D-2								+	+	+															2
Pyorrhea alveolaris	E	E					E								+	+	+															
Gingivitis	F	F	*Treponema microdentium*				F											+	+	+												
Vincent's infection	C	C-2					C-2											+	+	+												3
Pyorrhea alveolaris	G	G					G											+	+	+												
Vincent's infection Pernicious anemia	H	H					H														+	+										
Vincent's infection	T	T					T														+	+										4
Oral sepsis	K	K-1					K-1																+									5

Vincent's infection	C	C-3	Irregular, loosely coiled name?	No growth	+	−	C-3	+	6
Pyorrhea alveolaris	L	L	Large regularly coiled spirochetes *T. macrodentium*	+ hemolytic	−	+	L	+ + + +	7
Oral sepsis	K	K-2					K-2	+ + + +	
	Culture strains of *Treponema pallidum*	Reiter Kasan	Similar to strains L and K-2				Reiter Kasan	+ + + + + + + +	
		Nichols Noguchi	long, delicate, regularly coiled spirochetes	+ hemolytic	−	+	Nichols Noguchi	+ + + +	8
							NaCl		
							Mercapt. broth and blood		

+ = precipitate. The blank spaces indicate negative reactions

Immunization of animals. For the immunization of animals we have used suspensions of spirochetes in 0.85% NaCl solution containing 0.2% of phenol. The suspensions were prepared so as to contain approximately 100 spirochetes per darkfield (2.54 sq. mm.). This standardization of the vaccine has seemed to us preferable to a nephelometer standard, because the larger and coarser spirochetes will give the same turbidity with fewer organisms as will a larger number of smaller ones. In addition, suspensions of the organisms grown in blood broth might give a more pronounced turbidity due to the presence of blood cells or cell debris.

Rabbits were used exclusively as immune animals and we have used rabbit blood in our media for the preparation of vaccines or spirochetal extracts. Careful checks have been made to make certain that any immune reactions that occurred were not due to the formation of antibodies against the medium rather than to spirochetal antigen-antibody reactions.

The rabbits were immunized with a total of 11 cc. of vaccine, increasing doses being given over a period of three weeks. They were then bled 10 days following the last injection.

In several cases a satisfactory precipitating serum was obtained after one course of injections. In most cases however better sera were secured after a second or third course, one to six months after the first one. For these subsequent injections a total of 5 to 10 cc. of vaccine was given over a period of one to two weeks.

Spirochetal extracts. Spirochetal extracts have been used extensively for the possible sero-diagnosis of syphilis, but rarely for the differentiation of individual strains. Many of these extracts have been alcohol extracts of oral spirochetes and of nonpathogenic, cultivated *T. pallidum*, representing spirochetal lipoids more or less comparable to the non-specific lipoids of tissue extracts. Saline, aqueous and dialyzed aqueous extracts were also employed as well as trichloracetic acid extracts, tryptic digests, Becks F 68 fraction, culture filtrates, globulin solutions and extracts of globulin (2, 13, 14, 28–30, 33, 34, 36, 38, 44, 52, 71)

A number of attempts was made by us to obtain spirochetal extracts which would prove active and specific for certain strains or a group of strains of oral spirochetes. These attempts included hydrochloric acid extraction, trichloracetic acid extraction and lysis with sodium desoxycholate. These methods were abandoned in favor of the formamide extraction of spirochetes which we finally adopted.

Hydrolysis of spirochetes by hydrochloric acid did not yield serologically active extracts.

Addition of two drops of a 10% aqueous solution of sodium desoxycholate to 1 cc. of a slightly turbid spirochete suspension will lead to a macroscopic partial or complete clearing of the suspension Darkfield examination of macroscopically cleared suspensions shows disintegrated, but not completely lysed spirochetes.

Cleared spirochete suspensions and Seitz filtrates of such suspensions were added to different sera A heavy precipitate developed immediately or within a few minutes in almost all antispirochetal sera, in many normal rabbit sera and in a number of Wassermann positive and Wassermann negative human sera.

Identical results were obtained using instead of the spirochetal "lysate" a pancreatic digest broth or physiological salt solution to which aqueous sodium desoxycholate solution had been added in the same proportion. This indicated that the desoxycholate alone would precipitate undiluted sera. Rabbit sera in dilutions above 1:32 and human sera in dilutions above 1:8 did not give any precipitate with the sodium desoxycholate solutions, but sera in these dilutions also failed to react with spirochetal "lysate."

Extracts of spirochetes were prepared by us using the trichloracetic acid method of Boivin and Mesrobeanu (6). At the time that these extracts were tested we were unaware of Beck's work (2). We can confirm his results that trichloracetic acid extracts will precipitate spirochetal antisera, but fail to react with syphilitic sera. The extracts gave precipitates not only with the homologous spirochetal antisera, but also, even though to a lesser degree, with immune sera of morphologically or culturally different strains. The extracts may be of value as spirochetal antigens per se, but so far we have not been able to use them as a means of differentiation between various spirochete strains.

According to Eagle and Hogan (18) suspensions of cultured spirochetes remove from syphilitic serum all the antibodies both for the spirochetes and the tissue lipoids. It is therefore concluded that the antigenic factor of the spirochete which is serologically related to tissue lipoids is present on the surface of the organisms. It seems evident that spirochetes must contain antigenic components other than the surface lipoids and one would expect that dissolution of the spirochetal cell would make them more accessible to experimentation.

In the search for an organic solvent which would accomplish the complete dissolution of oral spirochetes our attention centered on a report by Fuller (21). Fuller reported formamide a suitable solvent for streptococci which had resisted solution in many other organic solvents.

Formamide is a colourless liquid which boils at 195°C. At a temperature of 150 to 160°C. it was found to be a good solvent for all spirochete strains thus far examined.

The addition of spirochetes to cold formamide will result in a heavily turbid suspension of the organisms. This suspension will clear after heating for 20 minutes at 150 to 160°C. Darkfield examination of such a solution will show little debris, but no intact spirochetes and no recognizable fragments of spirochetal cells.

Procedure of extraction. Spirochetes grown in mercapto-ethanol broth were centrifuged and washed with salt solution as outlined above. The yield of a flask of 500 cc. was approximately 1 to 1.5 cc. of packed organisms. To approximately 1 cc. of packed organisms were added 4 cc. of formamide. This heavy suspension was put into a large (25 to 40 cc.) pyrex tube and placed in a petrolatum bath, previously heated to 165°C. in an electric oven. Following this procedure the temperature dropped occasionally below 150°C. After the temperature had again reached 150°C. the spirochete suspension was left at 150 to 160°C. for 20 minutes,

removed from the oven and left to cool at room temperature. The resulting solution was always found to be clear or faintly opalescent.

To 4 cc. of "formamide solution" were added 10 cc. of acid alcohol (95 parts of absolute alcohol with 5 parts of 2 N HCl). The mixture was left at room temperature for 15 minutes and the resulting precipitate separated by centrifugation.

To the supernatant fluid were added 20 cc. of acetone (Merck Reagent). They were mixed well and left at room temperature for one hour. The precipitate was then spun down and the supernatant fluid discarded. The precipitate varied in amount which was sometimes considerable. It was washed twice with 5 cc. of acetone.

To the washed precipitate were added 6 cc. of 0.85% NaCl solution and the mixture was left at room temperature over night. In most instances only little of the precipitate went into solution. The remaining precipitate was removed by centrifugation.

To the supernatant fluid were added one drop of phenol red indicator and a trace of granular Na_2CO_3, enough to take the extract just beyond the neutral point. The resulting clear extract was used for precipitin tests with antispirochetal sera.

PRECIPITIN TESTS

During our experimental work we used a macro-method rather than a micro-precipitin test, because we found the results more convenient to read, especially if a large number of tests had to be read within one day. We used the ring test in Lancefield precipitin tubes (39) layering the extracts over equal parts of undiluted sera.

The results of these precipitin tests are recorded in Table I.

Depending on the quality of the sera and the concentration of the extracts precipitation occurred almost immediately, within 10 minutes, one hour, or as late as 4 to 5 hours later. All tubes were routinely kept over night, but only occasionally have we found a positive precipitation reaction in a tube that had not shown it within five hours. The precipitin tubes were kept at room temperature, after it was found that neither refrigeration nor incubation influenced the results.

It has been possible to dilute the formamide extracts prepared as described above, usually to 1:4 to 1:64, occasionally as far as 1:512. We have never encountered any extracts that were sufficiently concentrated to inhibit precipitation. There were no cross reactions between the eight serological groups.

Several of the extracts were stored in the refrigerator for as long as three months. Their activity after storage was found to be the same as when originally prepared.

As mentioned above, precipitation of the acid alcohol supernatant with acetone yields a rather heavy precipitate only a small part of which is soluble in salt solution at the slightly acid reaction. The remaining precipitate was found to be more soluble at pH 7 to 7.2, and almost completely soluble in salt solution at pH 8. It remained in solution if it was taken back to pH 7 to 7.2. Any of these three preparations gave a positive precipitin reaction with the homologous antiserum but also to a greater or lesser degree with all of our spirochetal immune sera. These extracts, apparently containing a substance common to all spirochetes examined, can therefore not be used for the differentiation of various strains by a precipitin test.

DISCUSSION

As shown in the table an active fraction has been extracted from 19 (out of 19) strains of oral spirochetes and from 4 (out of 4) strains of alleged *T. pallidum*.

Sixteen strains of oral spirochetes which were morphologically and culturally identical could be divided into 5 groups on a serological basis. None of these "groups" (1 to 5) gave positive precipitin reactions with the one strain (C-3) which was morphologically and culturally different from all others tested (group 6), with the larger oral spirochetes (group 7) or with the *pallidum* strains tested so far (groups 7 and 8). One strain (C-3) reacted only with its homologous antiserum (group 6).

Two oral spirochetes, strains L and K-2 were found serologically identical with the Reiter and Kasan strains of *T. pallidum* (group 7). These results correspond to their close morphological and cultural similarity. They were found serologically different from the smaller oral spirochetes and from the Nichols and Noguchi strains of *T. pallidum*.

The Nichols and Noguchi strains of *T. pallidum* were shown to be serologically related to each other, but serologically as well as morphologically different from the Reiter and Kasan strains; they were also serologically different from any of our strains of oral spirochetes. Kolmer, Kast and Lynch (35) found cross-reactions between the Nichols-Hough strain and the Noguchi strain on the one hand and *T. microdentium* and *T. macrodentium* on the other hand in agglutination and complement fixation tests. This raises the question as to

whether the precipitin test described is more specific than the agglutination and complement fixation tests, or whether the authors mentioned above used oral spirochetes serologically distinct from those employed by us.

It is still an open question whether these serological "groups" actually demonstrate the existence of different species, or whether they indicate the existence, of "sub-types" of one species as suggested by Vinzent and Séguin (69). The first interpretation is supported by the fact that this precipitin test differentiates morphologically dissimilar spirochetes. The latter interpretation is supported by the differentiation of morphologically identical organisms and by the rather large number of different "types" in the relatively small number of strains examined so far.

It is interesting to note that several strains isolated from the same patient on different occasions (A-1 to A-4, B-1 and B-2, D-1 and D-2) were identical according to the precipitin test. Other spirochetes isolated from the same specimen of one patient (K-1 and K-2, C-1, C-2 and C-3) were serologically different.

It is fully realized that the small yield of specific spirochetal extract from large numbers of spirochetes makes the test not suitable for routine work; but it is hoped that it may prove of value in the selection of spirochetes for experimental work or in the classification of these organisms.

SUMMARY

The inadequacy of our present means for identification and classification of oral spirochetes is pointed out.

A method is described by which specific extracts may be prepared from cultured spirochetes. The organisms are dissolved in hot formamide, treated with acid alcohol and acetone and the salt-soluble portion of the acetone precipitate is used as the antigen.

Various strains of oral spirochetes and cultured *T. pallidum* strains have been differentiated from each other using these extracts in a precipitin test with spirochetal immune sera.

BIBLIOGRAPHY

1. Akatsu, S.: J. Exp. Med., 1917, **25**, 375.
2. Beck, A.: J. Hyg., 1939, **39**, 298.

3. Bergey's Manual of Determinative Bacteriology, 5th Ed., 1939, Williams & Wilkins, Baltimore.
4. Besançon, E. and Etchegoin, E.: Compt. rend. Soc. biol., 1926, **95,** 475.
5. Blanchard, R.: Arch. de Parasitol., 1906, **10,** 129.
6. Boivin, A. and Mesrobeanu, L.: Compt. rend. Soc. biol., 1933, **113,** 490.
7. Brumpt, E.: Les spirochétoses. Nouveau traité de médicine, Paris, 1921–1922.
8. Castellani, A.: Lancet, 1906, I 1384.
9. Castellani and Chalmers: Man. Trop. Med., 1913, 2nd Ed., 402 and 1283.
10. Castellani, A.: J. Trop. Med. and Hyg., 1926, **29,** 360.
11. Cavalié, M. and Mandoul, A. H.: Compt. rend. Soc. biol., 1921, **85,** 1068.
12. Cohn, F.: Beitr. z. Biol. d. Pflanz., 1875, **3,** 199.
13. Craig, C. F. and Nichols, H. J.: J. A. M. A., 1912, **58,** 1163.
14. Craig, C. F. and Nichols, H. J.: J. Exp. Med., 1912, **16,** 336.
15. Daufresne, M. and Vinzent, R.: Compt. rend. Soc. Biol., 1938, **128,** 773.
16. Delamare, G.: Bull. soc. path. exot., 1926, **19,** 322.
17. Dobell, C. C.: Arch. f. Protistenk., 1912, **26,** 117–240.
18. Eagle, H. and Hogan, H. B.: J. Exp. Med., 1940, **71,** 215.
19. Fantham, H. B.: Ann. of Trop. Med. and Parasit., 1915, **9,** 391.
20. Fukushima, B.: Sci. Rep. Gov. Inst. Tokyo, 1927, **6,** 533.
21. Fuller, A. T.: Brit. J. Exp. Med., 1938, **19,** 130.
22. Gerber, P., Centralbl. Bakt. 1910, **56,** 508.
23. Hampp, E. G., J. A. D. A., 1943, **30,** 1066.
24. Hoffmann and Prowazek, Centralbl. Bakt., 1. Abt., 1906, **41,** 741 and 817.
25. Hoffman, E., Deutsch. med. Woch., 1920, **46,** 257.
26. Hoffmann, E.: Deutsch. med. Woch., 1920, **46,** 625.
27. Hoffmann, E., Centralbl. Bakt., Orig., 1921, **86,** 134.
28. Hoeltzer, R. R., and Popoff, W. J., Z. Immunitaetsforsch. 1928, **59,** 501.
29. Kast, C. and Kolmer, J. A., Am. J. Syph. Gon. Ven. Dis. 1940, **6,** 24.
30. Klopstock, F., Deutsch. med. Woch., 1926, **52,** 226.
31. Klopstock, F., Deutsch. med. Woch., 1927, **53,** 1261.
32. Koch, R., Beitr. z. Biol. d. Pflanz., 1877, **2,** 421.
33. Kolmer, J. A., Williams, W. W., Laubaugh, E. E., J. Med. Res., 1913, **28,** 345.
34. Kolmer, J. A., Kast, C. C., Lynch, E. R., Am. J. Syph. Gon Ven. Dis. 1941, **25,** 300.
35. Kolmer, J. A., Kast, C. C., Lynch, E. R., Am. J. Syph. Gon. Ven. Dis. 1941, **25,** 412.
36. Kolmer, J. A., Arch. Dermat. Syph. 1942, **45,** 455.
37. Kritchewski, B. and Séguin, P., Rev. de Stomat. 1920, **22,** 613.
38. Kroó, H., Schulze, F. O.: Klin. Woch., 1928, **7,** 246.
39. Lancefield, R.: Proc. Soc. Exp. Biol. Med. 1938, **38,** 473.
40. Miller, W. D.: Mikroorganismen der Mundhoehle. 1889.
41. Muttermilch, S., Séguin, P.: Compt. rend. Soc. Biol., 1923, **88,** 28.

42. Noguchi, H., J. Exp. Med., 1912, **15**, 81.
43. Noguchi, H., J. Exp. Med., 1912, **16**, 194.
44. Noguchi, H., J. A. M. A., 1912, **58**, 1163.
45. Noguchi, H., Akatsu, S., J. Exp. Med., 1917, **25**, 765.
46. Noguchi, H., The newer knowledge of bacteriology and immunology. Jordan and Falk. 1928, 481, University of Chicago Press.
47. Park and Williams, Pathogenic microorganisms. 1939, Lea and Febiger, Phila.
48. Pettit, A., Contrib. á l'Étude des Spirochétidés, Paris, 1928.
49. Plaut, H. C., Deutsch. med. Woch., 1894, **20**, 920.
50. Proske, H. O., Sayers, R. R., U.S. Publ. Health Rep., 1934, **49**, 839.
51. Pons, R., Bull. soc. path. exot. 1924, **17**, 170.
52. Rice, C. E., J. Immunol., 1932, **22**, 67.
53. Robinson, G. H., Am. J. Hyg., 1923, **3**, 483.
54. Rosebury, T., Foley, G., Proc. Soc. Exp. Biol. Med., 1941, **47**, 368.
55. Rosebury, T., Foley, G., J. Dent. Res., 1942, **21**, 308.
56. Sangiorgi, G., Pathologica revista, 1916, **8**, 283.
57. Séguin, P., Vinzent, R., Compt. rend. Soc. Biol., 1936, **121**, 408.
58. Smith, D. T.: Am. Rev. of Tbc., 1927, **16**, 584.
59. Smith, D. T.: J. Inf. Dis., 1930, **46**, 303.
60. Smith, D. T.: Oral spirochetes. Williams and Wilkins Co. 1932.
61. Strong, R. P.: Science, 1923, **57**, 514.
62. Veillon: Arch. de med. exper. d'anat. 1894, **6**, 161.
63. Vincent, M. H., Ann. Inst. Past., 1896, **10**, 488.
64. Vincent, M. H.: La presse mèd., 1898, **1**, 106.
65. Vincent, M. H.: Soc. mèd. des hôp., 1898, 244.
66. Vincent, M. H.: Lancet, 1905, **1**, 1260.
67. Vincent, M. H., Bull. Acad. de mèd. 1926, **96**, 135.
68. Vinzent, R., Daufresne, M.: Compt. rend. Soc. Biol., 1934, **116**, 490.
69. Vinzent, R., Séguin, P.: Ann. Inst. Past. 1938, **61**, 255.
70. Vinzent, R., Séguin, P.: Bull. de l'acad. med. 1939, **121**, 407.
71. Wadsworth, A. B., van Amstel, J. E., Brigham, M. W.: J. Immunol. 1930, **19**, 289.
72. Wichelhausen, O. W. and R. H.: J. Dent. Res. 1942, **21**, 543.
73. Zange, H.: Med. Klin., 1910, 29.

THE CONTROL OF EXPERIMENTAL PNEUMONIA WITH PENICILLIN

II. Comparison of the Intramuscular, Respiratory and Oral Routes of Administration, with Respect to Therapeutic Activity and Rate of Absorption

CATHERINE E. WILSON, CAROLYN W. HAMMOND, 1st Lt. CWS, ANN F. BYRNE, 2nd Lt. SnC

Medical Division, CWS, Edgewood Arsenal, Md.

AND

ELEANOR A. BLISS

The Johns Hopkins University, Medical School, Baltimore, Md.

The ultimate objective of these studies is the prevention of pneumonia in persons who have been exposed to irritant gases. They are concerned, therefore, not only with the prophylactic effects of various agents but also with the practicability of methods of administering them. Attention has been focused, in the main, on penicillin because the action of the sulfonamides in clinical and experimental infections is well known.

In a previous study it was shown (1) that, in lobar pneumonia in rats, as good results were obtained when penicillin was administered as an inhalant as when it was given by injection. While that work was in progress there began to appear in the literature reports (2, 3) to the effect that the peroral administration of penicillin, in large doses, yielded satisfactory blood levels in man and hence offered promise as another method of therapy. Recently, clinical trials have shown (4, 5, 6) that penicillin given by mouth is indeed successful in the treatment of certain diseases. Since therapy by the oral route would be simpler to apply than either injection or inhalation therapy it seemed that it would be advantageous to make controlled studies of the relative merits of these three ways of giving penicillin.

Little experimental work has been done with oral penicillin. In 1942, Powell and Jamieson (7) compared the effects of orally administered penicillin and sulfonamides on pneumococcal and staphylococcal peritonitis in mice. They found that ten 20 mg. doses of penicillin

(units not stated), given over a three day period, were more effective than five 30 mg. doses of sulfapyridine against the pneumococcal infection and gave better results than five 20 mg. doses of sulfathiazole in staphylococcal peritonitis. They made no comment on the use of the oral route for penicillin, although it was a distinct novelty at the time when they were writing. Schnitzer and his coworkers (8) reported, in abstract, that pneumococcal pneumonia in mice yielded as readily to oral as to parenteral therapy with penicillin, but larger doses were required per os.

In the present study rats, in which lobar pneumonia had been induced experimentally, have been treated with inhalations of aerosolized solutions, intramuscular injections and gastric intubations of penicillin. In the case of the larger doses the results of oral therapy have been compared with those obtained previously with parenteral therapy. In addition, studies have been made of the rate of absorption of penicillin into the blood stream from the three sites, in the hope of providing an explanation for certain differences which were noted.

METHODS

Production of Pneumonia. Lobar pneumonia was induced in rats by the intrabronchial intubation of mucin suspensions of type I pneumococci, according to the technique of Nungester and Jourdonais (9).

Therapeutic Procedures. The sodium salt of penicillin, obtained from several pharmaceutical companies, was used. For the two parenteral routes it was dissolved in M/50 phosphate buffer, pH 7.3. The injections (volume 0.2 cc.) were made into the muscles of the right hind leg. The technique of the aerosol inhalations was described in detail in the first report (1). Its essential features are exposure of only the snouts of the rats to the aerosol, determination of the concentration of the mist at the point of delivery and, from this and certain other factors, deriving an estimate of the amount of penicillin inhaled and retained by each animal, that is, an estimate of the actual dose of penicillin.

At the inception of this study belief in the importance of counteracting gastric acidity during oral administration of penicillin was current. For this reason several of the vehicles which had been suggested to circumvent destruction incident to oral administration were tested. At the same time it was realized that the rat does not present the same

problem in this respect that the human subject does. The rat has no free hydrochloric acid in its stomach whereas man has from 0.4 to 0.5 per cent free HCl. The pH of the excised rat stomach has been reported (10) to be 3.6 while that of the human stomach is 0.9 to 1.5 (11). The rat stomach evacuates a water-barium meal in about 2 hours. An ordinary mixed meal is evacuated by the human stomach in 3 to 4½ hours. Consequently, in the rat stomach penicillin is not subjected to as high a hydrogen ion concentration, nor does it remain in the stomach for as long a time as in the human stomach.

In our first experiments with oral therapy the penicillin was placed in the rats' food or drinking water. This method has proven valuable in administering sulfonamides to mice since it leads to a more or less regular drug intake and so to the maintenance of steady blood concentrations (12). It was unsatisfactory, however, in the present instance because the rats ate and drank little or nothing during the hours when they were recovering from ether anaesthesia and the shock of intrabronchial intubation. It was obvious that forcible feeding would have to be employed. The first vehicle tried was 4 per cent beeswax in peanut oil. This had been found effective by McDermott and his associates (3), following the initial observation of Libby (2) that oily suspensions of penicillin resulted in higher blood concentrations than aqueous solutions. In this vehicle, however, the penicillin evinced a strong tendency to settle out in the syringe and it was impossible to secure exact dosage.

The method finally selected was the administration by gavage of solutions of penicillin in phosphate buffer. The volume of each dose dose was 0.5 cc. The strength of the phosphate was increased to M/10, with the intention of lowering the gastric acidity, but the pH—7.3—was the same as that of the solutions for parenteral use. Food was withheld from the rats from the time of infection until after the final treatment. Since the first dose of penicillin was administered 6 hours after infection the rats were in a fasting state throughout the course of treatments. Libby (2) noted that better absorption occurred when oral therapy was given on an empty stomach and this has been confirmed by a number of investigators.

To provide a known standard of comparison for the oral therapy trials a group of mice was treated with sulfadiazine. The drug, finely

powdered and suspended in 5% acacia solution, was administered by gavage in 50 mg. doses.

Regardless of the therapeutic agent or the route of administration the schedule of therapy which was used in the first series of experiments was adhered to here, namely, three doses given at 6, 12 and 24 hours after the rats had been inoculated with the pneumococci. Three different amounts of penicillin were tested by each of the three routes. Data on the effects of 500 and 250 units via the intramuscular and respiratory routes were already in hand. Tests with 100 units per dose were now added. For oral therapy, because of the reports (13) that 5 to 10 times as much penicillin is required by this route to produce blood levels equivalent to those obtained following intramuscular injection, the doses which were administered by gavage were 5000, 2500 and 1000 units of penicillin.

Determination of Blood Concentration. Except as otherwise stated, normal rats were used. After a single treatment with penicillin, given by one of the three routes, the rats were anaesthetized with ether and bled from the heart. The first blood was taken at 15 minutes after the oral or intramuscular administration of penicillin or, in the case of aerosolization, as soon as possible after cessation of treatment—that is at 5 or 6 minutes. Later bleedings were made at ½ hour, 1 hour and 2 hours after administration. The rats were re-anaesthetized for each bleeding. For the first three bleedings 0.2 cc. of blood was withdrawn; for the last 0.4 cc. was required. The needles and syringes were moistened with heparin solution to delay clotting of the blood. They were thoroughly rinsed with physiological salt solution between bleedings and again moistened with heparin. The rats, which were studied in groups of 5 to 8, were marked and followed individually.

The penicillin content of the blood was determined by the test-tube method of assay, using blood cells and a strain of haemolytic streptococcus. The technique is approximately that of Rammelkamp (14). For the details of the method see Appendix A.

RESULTS

The results of the therapeutic tests are shown in Table 1. Five per cent of the untreated animals survived. Three 50 mg. doses of sulfadiazine protected 67 per cent of the rats, while penicillin protected 36

TABLE 1

Effect of Penicillin upon Pneumonia in Rats, According to Dose and Route of Administration. Effect of Sulfadiazine Given by Mouth

DRUG	TREATMENT		NO. OF RATS	RESULTS									
	Route	Dose per treatment*		No. dying	Deaths on day								Per cent surviving
					1	2	3	4	5	6	7	later	
Sulfadiazine in 5% acacia	Oral (gavage)	50 mg.	30	10			1	1				8	67
Penicillin in M/10 Phosphate Buffer	Oral (gavage)	1000 units	20	16	1	3	4	6			1	1	36
		2500 units	30	11			1	3	1	2	1	3	63
		5000 units	25	1								1	95
Penicillin in M/50 Phosphate Buffer	Intramuscular (injection)	100 units	25	14		1	3	4	3		1	2	44
		250 units	28‡	15		1	2	9		1		2	46
		500 units	28‡	0									100
Penicillin in M/50 Phosphate Buffer	Respiratory (inhalation)	100† units	22	10	2	1	4	3					55
		250 units	36‡	9				6		1		2	75
		500 units	39‡	1						1			97
None			41	39		15	9	9	2	2		2	5

* 3 treatments were given—at 6, 12 and 24 hrs. after infection.

† The values for the doses administered as an aerosol are only an approximation.

‡ Previously reported.

TABLE 2

Penicillin Blood Levels in Rats, According to Route, Dose and Time

ROUTE	DOSE	TIME AFTER COMPLETION OF EX	NO. OF RATS	UNITS OF PENICILLIN/CC. OF BLOOD										GEOMETRIC MEAN UNITS/CC.	
				2.0	1.0	.5	.25	.125	.062	.031	.015	<.03	<.015		
	units	*min.*		*no. of rats*											*log*
Inhalation	500	5	8	1	5	2								.92	$\bar{1}$.96
		30	8			4	3	1						.32	$\bar{1}$.50
		60	8				2	5	1					.136	$\bar{1}$.13
		120	8					1	3	1	3			.036	$\bar{2}$.56
Intramusc.	500	15	5			2	3							.33	$\bar{1}$.52
		30	10		1	3	6							.354	$\bar{1}$.55
		60	10			2	1	4	3					.142	$\bar{1}$.15
		120	10			1				2	3		4	.02	$\bar{2}$.30
Oral	5000	15	11		4	2	2	3						.39	$\bar{1}$.59
		30	11	1	2	3	2	3						.38	$\bar{1}$.58
		60	11	3		2	3	2	1					.39	$\bar{1}$.59
		120	11		1	3		4	3					.18	$\bar{1}$.25
Inhalation	250	5	8		1	3	4							.39	$\bar{1}$.59
		30	8				1	6	1					.124	$\bar{1}$.09
		60	7					1	6					.067	$\bar{2}$.83
		120	8						1	2	1		4	.013	$\bar{2}$.11
Intramusc.	250	15	6				1	5						.140	$\bar{1}$.15
		30	16				3	10	3					.124	$\bar{1}$.09
		60	15				1	6	6	2				.081	$\bar{2}$.91
		120	15							1	3		11	not determinable	
Oral	2500	15	15			1	6	5	3					.159	$\bar{1}$.20
		30	15				1	5	4	3	1			.136	$\bar{1}$.13

Oral	2500	15	15			1	6	5	3					.159	$\bar{1}.20$
		30	14				5	5	4					.136	$\bar{1}.13$
		60	15				2	3	8			2		.071	$\bar{2}.85$
		120	15					1	1		2	7	4	not determinable	
Inhalation	100	5	5				2	2	1					.131	$\bar{1}.12$
		30	5					2	1	2				.062	$\bar{2}.79$
		60	5						2	1	1		1	.026	$\bar{2}.42$
		120	5						1	1			3	not determinable	
Intramusc.	100	2	10			1	2	2	4	1				.11	$\bar{1}.04$
		15	5				4	1						.22	$\bar{1}.34$
		30	15					5	6	3	1			.08	$\bar{2}.90$
		60	14					1	2	3	2		6	.03	$\bar{2}.48$

to 100 per cent depending upon the dose and the route of administration.

The data on penicillin indicate that approximately 10 times as much of the agent is required by the oral as by the parenteral routes. In order to arrive at a more exact evaluation of the amounts required by the three routes, the median survival doses were calculated by the method outlined in Appendix B. These were found to be 1585 units per treatment for oral therapy, 174 units for intramuscular therapy and 119 units for inhalation therapy. The ratios between oral and intramuscular therapy and oral and inhalation therapy are 9.1 to 1 and 13.3 to 1, respectively. The differences between the oral and parenteral median survival doses are, of course, statistically significant. The ratio between intramuscular and inhlation therapy is 1.4 to 1 and here the difference is not significant. ($P = .0536$)

In Table 2 and Fig. 1 are shown the results of the blood studies. The Table has been arranged to convey an idea of the individual variation. It is seen that the readings for the two parenteral routes, at the first two or three periods, cover a fairly narrow range of concentrations while those for oral administration are more widely distributed. The mean (geometric) blood concentrations and their logarithms are shown in the last two columns. The latter are used as the ordinates in Fig. 1 which gives a better perception than the tabulated material of the amount of agreement between the levels obtained following the three routes of administration.

In plotting the data, the time from which to date administration of the aerosol raised a problem. Aerosolization is not an instantaneous operation; here, it extended over a period of 20 minutes. Absorption into the blood stream probably begins almost immediately but the full dose is not taken in until the end of the period. The best solution seemed to be to date the levels for this route from midway through the treatment time. In a small series of rats aerosolization with a 500 u/liter mist was stopped at 10 minutes and the penicillin content of the blood was determined immediately. It ranged from .25 to .125 u/cc. with a mean of .218 u/cc.—values considerably lower than half those observed following the full 20 minute exposure. Nevertheless, in Fig. 1, the mean level of blood taken 5 minutes after cessation of aerosolization has been plotted as the 15 minute point and the 30 minute bleeding as 40 minutes and so on.

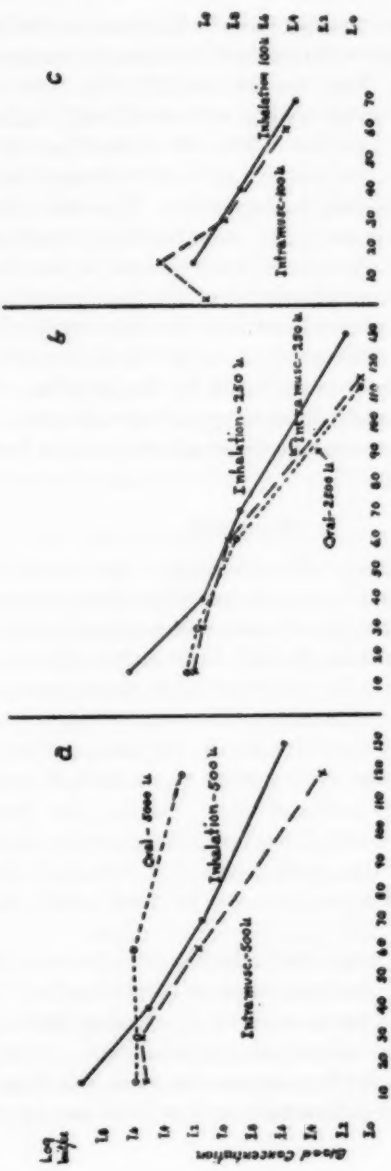


FIG. 1. BLOOD CONCENTRATION-TIME CURVES FOR PENICILLIN GIVEN BY DIFFERENT ROUTES
(Time starts with administration except in the case of inhalation, where it dates from halfway through the treatment period.)

There is a fair agreement between the blood concentrations obtained with the intramuscular and respiratory routes of administration at all three dosage levels. With the 500 and 250 unit doses of penicillin (Fig. 1, a & b), the initial reading was considerably higher following inhalation than after injection. With 100 units (Fig. 1 c) the concentration following injection rose to a peak at 15 minutes which is higher than the 15 minute reading for inhalation. It is also higher than the 15 minute reading following 250 units injected intramuscularly. It should be noted that the rate of disappearance of penicillin from the blood is in general faster following injection than following inhalation.

There is a marked difference between the two curves for oral administration. Following 2500 units per os the blood concentrations were almost identical with those obtained by the injection of 250 units. With 5000 per os, a totally different type of curve resulted. The mean level remained almost constant for 60 minutes and at two hours was still high.

DISCUSSION

The results of therapy with sulfadiazine were much as had been anticipated. Kepl and Gunn (15) using the same technique for producing type I pneumonia in rats were able to protect from 17 to 55 per cent of the animals with single daily doses of 36 to 200 mg. of sulfapyridine. One would expect somewhat better results with sulfadiazine, and with more intensive therapy at the start. A higher survival rate would no doubt have been obtained in the present series had dosage with either sulfadiazine or the smaller amounts of penicillin been continued for a longer period of time. The fact that the number of deaths which occurred after the seventh day is greater in the sulfadiazine than in the penicillin group presumably reflects the slower excretion of the sulfonamide and, perhaps, the more purely bacteriostatic nature of its action.

In considering the date relative to the ratios between the doses of penicillin required by the three routes of administration, it should be borne in mind that the median survival doses, especially the one for the intramuscular route, are only rough approximations. At the same time the observations recorded here support the view, arrived at in patients on the basis of blood concentrations, that from five to ten times as

much penicillin is required for oral as for parenteral therapy. In the only previous experimental study of the ratio between therapeutically effective doses of oral and parenteral penicillin, Schnitzer and his associates (8) found that it was 10 to 1 in mice with streptococcal and pneumococcal peritonitis. In certain other infections, however, they obtained a much lower ratio.

It seemed reasonable to assume that the differences between the doses required by the three routes were caused by differences in the rate of absorption of penicillin from the three sites. This assumption is not fully borne out here. Where the greatest difference in absorption was noted—with the 500 unit parenteral doses and the 5000 units per os—there was the least difference in therapeutic results. In this case, however, the survival rate was almost 100 per cent for all three routes and it is possible that the optimum dose had been exceeded. At the 250/2500 unit dosage level, the inferior results obtained with intramuscular administration cannot be ascribed to differences in blood concentration.

When penicillin was administered by mouth the concentration in the blood rose to its highest point in most instances within 15 minutes, as is shown in the concentration-time curves. In following the individual rats this condition was found to hold for 20 of the 26 animals which received the drug by this route. In 13, the highest level was maintained for 30 minutes and in 6, for 1 hour. The peak first occurred at 30 minutes in 3, and in 3 it was delayed for 60 minutes. On the whole, absorption was faster and, in the case of the 5000 unit dose, more complete than was to have been expected from the studies of others on human beings.

One would expect soluble substances to be absorbed from the lungs with extreme rapidity because of the large surface involved. Indeed, Geiser, Schaub and Staub (16) assert that giving penicillin via the respiratory route is tantamount to administering it intravenously. The observations made in the present study appeared at first to support this view. The peak blood concentrations occurred earlier and, in two of the three series, were higher in rats receiving penicillin as an inhalant than in those given intramuscular injections. But it was puzzling to find that the blood concentrations were also better maintained in the aerosolized than in the injected animals. It is hard to understand how

one of the routes could excel the other in both rapidity of absorption and maintenance of blood level for, once in the blood, the rate of elimination should be the same in the two instances. Two explanations suggest themselves. One is that the dose of the inhalant was larger than had been calculated and hence that the high initial level was produced by the presence of a larger amount of penicillin rather than by faster absorption. The other possibility which comes to mind is that the rapid decline in blood levels following intramuscular injection was caused by a deterioration of penicillin *in situ*, with the result that only part of that which was absorbed was detectable by the bioassay method

In considering the findings presented here it should be remembered that the chance of error is great in all work with penicillin. Crystalline penicillin was not available in sufficient quantity for a study of this sort and the preparation used here was the commercial sodium salt. It has been reported (17) that an ampoule marked 100,000 units may contain anywhere from 85,000 to 120,000 units. More important, the content of active penicillin may vary. Then there is the great error which is inherent in the two-fold dilution method of assay. In addition to the errors attributable to penicillin, there is also the uncertainty regarding the dose administered by inhalation (1). In short, it is unwise to overemphasize certain of the differences noted here in therapeutic results and in blood concentrations. The difference between the doses required via the oral and the parenteral routes is clear cut.

CONCLUSIONS

1. Type I pneumococcal pneumonia, experimentally induced in rats, can be controlled by the oral administration of penicillin.

2. In order to obtain equivalent survival rates, the amount of penicillin which had to be given by mouth was nine times that needed for intramuscular injections and thirteen times that needed for inhalation therapy.

3. The blood concentration-time curves following the administration of equivalent doses of penicillin by the intramuscular and pulmonary routes were much alike. Levels following 2500 units given by mouth were similar to those following 250 units by the two parenteral routes. Oral administration of 5000 units resulted in a different type of curve.

Appendix A. Penicillin Assay for Small Amounts of Blood

A series of small Wassermann tubes is set up, into each of which is pipetted 0.2 cc. of a 2½ per cent suspension of washed, citrated rabbit erythrocytes in saline. The first tube receives 0.2 cc. of rat blood and from it serial two-fold dilutions are made. (Blood from the last bleeding is placed in an empty tube as well as into one containing the diluent.) The tubes are then inoculated with 0.2 cc. of a 1:1000 brain heart broth dilution of the C203 strain of haemolytic streptococcus, 18–20 hours old. A standard solution of penicillin, diluted in saline to contain 1.0 unit per cc. is serially diluted in the red cell-saline suspension and inoculated with the same amount of streptococcus culture. After 18 hours ncubation the tubes are examined for haemolysis and turbidity and the amount of penicillin n the unknown is estimated by comparison with the standard. Ordinarily the end-points

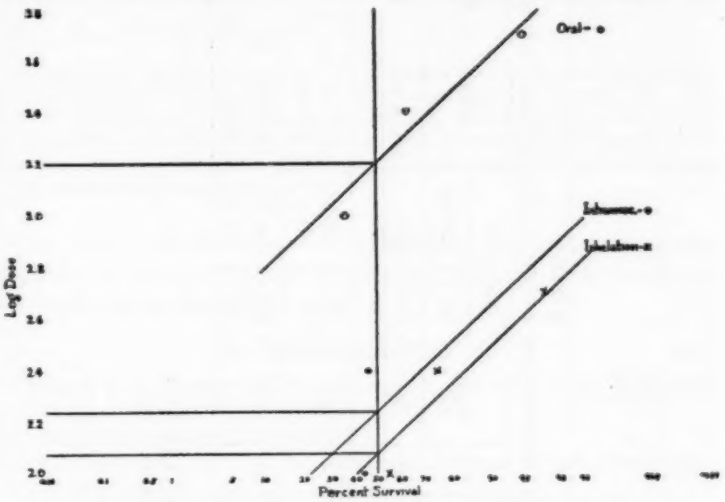


FIG. 2. DOSAGE-SURVIVAL CURVES FOR THREE ROUTES OF ADMINISTRATION OF PENICILLIN

are clear cut. Confusion is most apt to occur when the end-point falls in the first two or three tubes where there is a heavy concentration of rat blood. In that event subcultures are made on blood agar plates and estimation is deferred another 24 hours.

The first tube of the standard to show growth is usually that containing the 1:128 dilution—.007 unit per cc. Thus the smallest concentration of the unknown that can be detected is .015 unit per cc. The concentration frequently was lower than this in the two-hour readings and made calculation of the mean blood concentration difficult. The geometric mean is the logical constant to use with geometric series of observations and was employed here, but it does not permit of zero readings. To get around this difficulty, readings of less than .015 unit per cc. were called .007 unit, when the majority of the rats showed levels of .015 unit or above. When the majority of the readings were below .015 unit the mean was not calculated.

TABLE 3

Data Used in Calculating Standard Errors and Ratios of SD_{50} for Three Routes

ROUTE	SD_{50}* (log)	SD_{50}* (absolute)	σ_{tad} (log)	"n"†	$SE_{SD_{50}}$ (log)	$SD_{50} \pm 2S.E.$ (log)	$SD_{50} \pm 2S.E.$ (absolute)	ROUTES	DIFF. BETWEEN SD_{50} (log)	S.E. OF DIFF.	P‡	RATIO BETWEEN SD_{50} (ABSOLUTE)
Oral	3.2	1584.9	.32	55	.061	3.078–3.322	1197–2099	Oral & Resp.	1.125	.085	.0001	13.3
Intramusc.	2.24	173.8	.32	53	.062	2.116–2.364	130.6–231.2	Oral & Intramusc.	.96	.087	.0001	9.1
Respiratory	2.075	118.9	.32	58	.059	1.956–2.193	90.4–156.3	Intramusc. & Resp.	.165	.086	.0535	1.5

* From Fig. 2.

† Number of rats included in calculation of S.E.

‡ Probability of difference arising by chance.

Appendix B. Calculation of Median Survival Dose and Its Standard Error

The median survival doses (SD_{50}) were determined by plotting percentage survival against log of dose (per treatment) on Arithmetic-Probability paper. The experiments on intramuscular injections furnished virtually no evidence as to the slope of the dosage-survival curve, since 100 units and 250 units gave nearly identical survival rates and 500 units resulted in 100 per cent survivals. Because of the fact that the curves for the other two series plotted (Fig. 2) as parallel lines on probability paper, it was assumed that the intramuscular series was also parallel to these two. The validity of this assumption was checked by the survival indicated by this line at 500 units—26 out of 28 animals instead of 28 out of 28. Since this difference is within sampling variation the assumption was considered reasonable and this line was used for estimating σ_{ind}. The Standard Error of the SD_{50} was calculated by the method of Litchfield and Fertig (18), whose equation is: S.E.-$\frac{\sigma_{ind}}{\sqrt{n/2}}$. The data are shown in Table 3.

The parallelism between the three lines in Fig. 2 means that the increase in survivorship for a given multiple of dose was the same for all three routes of administration. The values of the $SE_{SD_{50}}$ are interpreted as showing that the chances are 21 to 1 that the *true* median survival doses lie between 90 and 156 units of penicillin (per treatment) for inhalation therapy, between 131 and 231 units for intramuscular therapy and between 1197 and 2099 units for oral therapy.

The authors are indebted to Dr. Margaret Merrell of the Johns Hopkins University, School of Hygiene and Public Health, for help in the statistical analysis of the data.

BIBLIOGRAPHY

1. Wilson, C. E., Hammond, C. W., Byrne, A. F. and Bliss, E. A.: Bull. Johns Hopkins Hospital **77**, 411, 1945.
2. Libby, R. L.: Science **101**, 178, 1945.
3. McDermott, W., Bunn, P. A., Benoit, M., DuBois, R., and Haynis, W.: Science **101**, 228, 1945.
4. Bunn, R. A., McDermott, W., Hadley, S. J., and Carter, A. C.: Jour. Amer. Med. Assoc. **129**, 320, 1945.
5. Finland, M., Mead, M., and Ory, E. M.: Jour. Amer. Med. Assoc. **129**, 315, 1945.
6. Ross, S., Burke, F. G., and McLendon, P. A.: Journ. Amer. Med. Assoc. **129**, 327, 1945.
7. Powell, H. M., and Jamieson, W. A.: Proc. Soc. Exper. Biol. and Med. **49**, 327, 1942.
8. Schnitzer, R. J., Ercoli, N., Longacre, J. D., and Soo-Hoo, G.: Bull. N. Y. Acad. Med. **21**, 447, 1945.
9. Nungester, W. J., and Jourdonais, L. F.: Jour. Infec. Dis., **59**, 258, 1936.
10. Machella, T. E., and Griffith, J. Q., Jr.: *The Rat in Laboratory Investigations*, Edited by Griffith and Faris, J. B. Lippincott Co., Philadelphia, p. 156, 1942.

11. BEST, C. H., AND TAYLOR, N. B.: *The Physiological Basis of Medical Practice*, 3rd edition, The Williams & Wilkins Co., Baltimore, pp. 710, 813, 1945.
12. LITCHFIELD, J. T., JR., WHITE, H. J., AND MARSHALL, E. K., JR.: Jour. Pharm. and Exp. Ther. **67**, 437, 1939.
13. KEEFER, C. S.: Jour. Amer. Pharm. Assn. **6**, 210, 1945.
14. RAMMELKAMP, C. H.: Proc. Soc. Exp. Biol. and Med. **51**, 95, 1942.
15. KEPL, M., AND GUNN, F. D.: Proc. Soc. Exp. Biol. and Med. **40**, 258, 1939.
16. GEISER, P., SCHAUB, K., AND STAUB, H.: Schweiz. Med. Wchnschr. **76**, 285, 1946.
17. KNUDSEN, S. F., AND RANDALL, W. A.: Jour. Bact. **50**, 187, 1945.
18. LITCHFIELD, J. T., JR., AND FERTIG, J. W.: Bull. Johns Hopkins Hosp. **69**, 276, 1941.

PROCEEDINGS OF THE MEETINGS OF THE JOHNS HOPKINS MEDICAL SOCIETY

Held in Hurd Memorial Hall, March 11, 1946

Inhalation Therapy of Experimental Pneumonia in Rats. CATHERINE E. WILSON, LT. CAROLYN W. HAMMOND, LT. ANNE F. BYRNE and ELEANOR A. BLISS (*Chemical Warfare, Edgewood Arsenal,* and *Department of Preventive Medicine, School of Hygiene.*)

In addition to the results published in the *Bulletin of The Johns Hopkins Hospital* (Dec. 1945), which showed that equivalent doses of penicillin were equally effective in the control of experimental pneumonia whether they were given by injection or as an inhalant, data on the absorption of penicillin were reported. Blood concentration-time curves following inhalation of aerosolized solutions closely approximated those obtained after intramuscular injections of penicillin. The main difference was in the more rapid absorption which occurred after inhalation. From this it was concluded that administration of penicillin as an inhalant was simply another way of introducing it systemically rather than a means of securing local action.

DISCUSSION

Dr. Horace L. Hodes: I should like to ask what factor is involved in swallowing penicillin. What takes place?

Dr. Bliss: I don't know. We think the rats do not swallow very much. Of course, it takes about five or ten times as much penicillin by mouth to get the same effect as by inhalation.

Dr. Hodes: That is the point I was trying to raise. If you got the same level by inhalation as by injection, despite the loss of penicillin by swallowing it would seem that inhalation is more effective than injection.

Dr. Bliss: Our mist was fine and so comparatively little stuck to the throat. We tried our best to fix it so that the rats could breathe only through the mouth or the nose, but that could not be arranged.

Student: I should like to ask Dr. Bliss if she were able to determine the concentration of penicillin in the lung, perhaps at autopsy?

Dr. Bliss: This we did not do. The people at Coldspring Harbor tried that, and they reported that there was a trace.

Dr. A. Murray Fisher: I thought Dr. Bliss said that the penicillin level in the blood went up to its peak after inhalation rather quickly? Is that right? Did you take blood at five minutes after inhalation?

Dr. Bliss: The peak blood level after inhalation was within the first six minutes. We did not determine the blood level at five minutes after intramuscular injection because the 30 minute reading was higher than the 15 minute one, indicating that the concentration was on the way up at 15 minutes.

Recent Experimental Studies on Aerial Disinfection with Glycol Vapors. O. H. ROBERTSON (*Department of Medicine, University of Chicago*).

In a brief review of the early work on the germicidal action of glycol vapors on bacteria floating in the air, it was pointed out that the killing of such bacteria depends on the rapid condensation of glycol molecules on the moist bacterial particle. When the concentration of glycol around the bacterium reaches 70 to 80 per cent, which may occur in a matter of seconds, it becomes bactericidal. In the case of propylene glycol this effect is almost instantaneous. Influenza virus sprayed into the air is also highly susceptible to the lethal action of glycol vapors. The two glycols principally studied are propylene glycol and triethylene glycol since they both possess marked bactericidal properties and have been shown to be relatively non-toxic. Prolonged tests on animals exposed constantly to vapors of these two glycols for over a year showed no deleterious effects. Although triethylene glycol does not exert its lethal action on bacteria and influenza virus as rapidly as does propylene glycol, for practical application it has many advantages over the latter, particularly that it is about 100 times as potent.

Certain studies on the application of glycol vapors to the control of airborne infections have been undertaken during the past several years by the Commission on Air-Borne Infections of the Army Epidemiological Board. As an essential preliminary to such studies it was necessary to devise suitable apparatus for dispersing triethylene glycol vapor and for controlling its concentration in the air. Development of the latter device—a "glycostat"—has been particularly useful, since in addition to being able to control the delivery of vapor into a room in much the same way that a humidostat controls the supply of water vapor in an airconditioned space, this instrument has shown us that the per cent saturation of the air with glycol vapor is a most important consideration in determining its germicidal activity.

Studies carried out in army hospital wards housing patients suffering upper respiratory streptococcal infection showed that the large numbers of hemolytic streptococci constantly present in the air of these wards could be reduced by 95 per cent when triethylene glycol vapor was introduced in bactericidal concentrations. Such amounts of glycol were not detectable by the occupants of the wards. Best results were obtained by combining dust control measures with the glycol.

Practical application of this method of aerial disinfection for the purpose of prevention of air borne respiratory disease has only just begun. Two such studies

carried on by other workers have shown a decided reduction of acute respiratory infection in the areas treated with glycol. A more comprehensive test of the prophylactic effect of triethylene glycol vapor is now being conducted under the auspices of the Commission on Air-Borne Infections in two of Dr. Edwards Park's infants wards of the Harriet Lane Home. While it is too early to give exact data on the difference in the incidence of cross infections occurring in the glycol-treated and control wards it can be stated that many fewer such infections are occurring on the glycol-treated ward.

DISCUSSION

Dr. William P. Longmire: Has this method been used in operating rooms?

Dr. Robertson: No, there has not as yet been any apparatus available for this kind of work. Such apparatus must be reliable and fairly simple. As yet, it is in the experimental stage; but it is now being made and I think before long it will be available. It will take a lot of observation, however, to indicate the usefulness of glycol vapors.

Major Clayton G. Loosli: Do you care to comment on the possibility of the use of other compounds, such as lactic acid?

Dr. Robertson: There have been studies on other types of compound. One of the most recent, as Major Loosli says, is lactic acid. That work was done in England where they could not get triethylene glycol and wanted something which had a chemical structure similar to that of glycol. They found it very effective. Other compounds have been tried also—sodium hypochlorite, hexylresorcinal, for instance—but there are certain objections to all of these.

Dr. A. Murray Fisher: I would like to ask about humidity. I think Dr. Robertson said that if the humidity is high, the killing power is cut too low and that this is also true when it is very low. I wonder what the optimum humidity is.

Dr. Robertson: There are two aspects to this question—the effect on moist droplets and the effect on dry particles. Actually, when you have moist particles containing bacteria, you can get bactericidal effects at very low humidities if you have enough glycol. Now when you have dry particles (I wish I could be more specific about this, but we have not studied them sufficiently), the effect is not nearly as great at low humidity. I presume this is because not enough moisture collects on them. These are problems which need solution. Actually if you put glycol in a room, you will not kill all the bacteria. It is not possible, under the present state of our knowledge, to sterilize completely the air of a room such as this.

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal)

Traumatic Injuries of Facial Bones. By JOHN B. ERICH and LOUIS T. AUSTIN. Illus. 600 pp. $6.00. *W. B. Saunders Company, Philadelphia, Pennsylvania,* 1944.

The management of a wide variety of facial fractures is presented by the authors in a clear, concise manner. The material is arranged in handbook form with a complete discussion of a specific type of fracture and the recommended treatment in one section so that the necessary information for the treatment of almost any fracture or combination of fractures of the facial bones is readily accessible in a page or two.

Methods of treatment recommended are the procedures that have produced the most satisfactory results in the personal experience of the authors. They have made no attempt to review or to comment upon the numerous other methods of treatment of facial fractures to be found in the literature.

The book is handsomely printed. The numerous exceptionally fine photographs of skeletal moulages and actual cases aid in the clear visualization of the type of fracture discussed and the corrective measures applied.

This book can be highly recommended to everyone interested in traumatic surgery.

W. P. L.

Narco-Analysis. By J. STEPHEN HORSLEY. 134 pp. $2.50. *Oxford University Press, New York, New York,* 1946.

There are many physicians who have used barbiturate interviews or observed their effects in the treatment of acute war neuroses. They will find much of historical interest in this recent American edition of a 1943 English book. They will find here, also, much that may have been missed in the hurried experiences of the war—an effectively written exposition of psychotherapeutic principles. The author gives a careful account of what may be called the tactical uses of narco-analysis in a soundly conceived strategy of psychotherapy, both for psychiatric first-aid and as a tool in deep therapy.

The author strongly recommends the use of the drug-analytic method by the inexperienced medical officer called upon to treat acute psychoneuroses in the battlefield. Your reviewer cannot endorse this recommendation (made in 1943) for it appears that some basic training in psychotherapy is needed for its safe and effective use.

Your reviewer, however, enthusiastically recommends this book to all physicians for its unusually clear exposition of psychotherapeutic principles.

J. C. W.

Surgical Treatment of the Nervous System. By FREDERIC W. BANCROFT. Illus. 534 pp. $18.00. *J. B. Lippincott, Philadelphia, Pennsylvania,* 1946.

This volume, devoted to operative neurological surgery, is one of a series dealing with various aspects of general surgical treatment under the Supervising Editorship of Frederic W. Bancroft. Its seventeen authors are men pre-eminent in their field.

The book is arranged in seven sections of which three deal with cranial and intracranial surgery. These are (1) Congenital, Traumatic and Inflammatory Disorders, (2) Tumors, (3) Cranial Nerve Disorders, Involuntary Movements and Epilepsy. The remaining four sections are devoted to Spinal Cord, Peripheral Nerves, Sympathetic Nervous System, and Chemotherapy. Dedication is to the memory of Harvey Cushing and, as the preface points out, "through the pages of this volume, the invisible hand of Harvey Cushing makes known its omnipresent influence." This is only too often true, as in the advocacy of wet drapes in the operating room, and a drainage of clean wounds.

There are a number of excellent chapters. The one on Craniocerebral Trauma, by R. Glen Spurling, is a sound, concise and eminently practical discussion of the problem. Tumors of the cranial nerves are also well handled by W. McK. Craig and C. Hunter Shelden. The chapter on Tumors of the Hypophyseal Region, also by Craig, is excellent except for inadequate handling of the subject of aneurysms of the Circle of Willis. Another outstanding chapter is that on Surgery of Disorders of Cranial Nerves, by Max M. Peet and Dean H. Echols. The fairness of these authors in evaluating the subject of temporal vs. occipital approach for section of the trigeminal nerve is remarkable in view of their known and extraordinary facility with the former.

The section on the Spinal Cord, by Howard C. Naffziger and Edwin B. Boldrey, is good, except that the discussion on treatment of ruptured intervertebral disks is dated. The authors fail to stress the multiplicity of disks, knowledge of which is of great importance in successful treatment. They are also excessively impressed with the importance of the ligamentum flavum alone in the production of symptoms.

The handling of Peripheral Nerves, by Claude C. Coleman, and the section on the Sympathetic Nervous System, by James C. White, are complete and well suited to such a volume. Erickson's chapter on Epilepsy due to Atrophic Cerebral Lesions is excellent, but perhaps there should have been a little broader discussion of epilepsy for this volume.

The chapter on Infantile Hydrocephalus is interesting because of a statement that block of the Aqueduct of Sylvius is rare as a cause of hydrocephalus in infants (actually nearly 50%), and also because of an expression of some doubt as to the

actual mechanism of hydrocephalus. As pointed out by Dandy many years ago, the cause and mechanism can always be grossly demonstrated.

The book as a whole makes for good reading. It combines in one volume the opinions of numerous outstanding neurosurgeons, but the inexperienced reader may find it difficult to separate the good from the bad, or indifferent.

F. J. O.

Modern Methods of Amputation. By EDMUND VASCONCELOS. Illus. 253 pp. $10.00. *The Philosophical Library, New York, New York,* 1946.

This is a good practical presentation of a very important and, to some extent, controversial subject. The introductory historical survey by General Kirk is excellent. The book is divided into six chapters. In the first chapter is a general discussion of methods and levels of amputation in the upper and lower extremities. In the second, the author presents the general principles of surgical technique and of pre- and postoperative care. The indications for amputation are next considered. The fourth and fifth chapters present the technique for various types of amputations and are beautifully and simply illustrated by the drawings of Jose Goncalves Filho. Finally the protheses are discussed.

As in any subject about which opinions vary so decidedly, the reader will perhaps encounter a number of statements and suggestions with which he will disagree. Altogether, however, the author has done a splendid job of sifting out unessential and undesirable methods and has made a useful presentation which students and practitioners will find helpful.

It would probably have served no useful purpose if the publishers had reedited the book into more idiomatic English. It is to be regretted, however, that a few ill chosen words and phrases which momentarily break the reader's train of thought were not properly corrected.

H. B. S., JR.

Preoperative and Postoperative Treatment. Edited by LT. COL. ROBERT L. MASON and HAROLD A. ZINTEL. 2nd Ed. Illus. 584 pp. $5.00. *W. B. Saunders Co., Philadelphia, Pennsylvania,* 1945.

When this excellent monograph first appeared in 1937 it filled an important need in the surgical literature. The authors have now thoroughly revised the book, added a number of chapters, and have succeeded in incorporating into it the many new advances which have been made in recent years. No surgeon can be unaware of the great importance of preoperative and postoperative care in regard to the morbidity, mortality, and general well-being of his patients. Here has been assimilated in a delightful and informative way the best surgical thought upon all aspects of the subject. The physiological, biochemical, bacteriological, and pharmacological aspects of these problems are discussed. An excellent brief bibliography is appended to each chapter. The scientific background of the various physiological changes which may occur and require attention before or after operation is presented and at the same time the discussion loses nothing of its

practicality by this method but, on the other hand, gains in clarity. One may naturally find here and there some minor points with which he will not be in agreement. Every interested reader will find the book stimulating and helpful. It should be included in every surgeon's library.

H. B. S., Jr.

Facial Prosthesis. By Arthur H. Bulbulian. Illus. 241 pp. $5.00. *W. B. Saunders Company, Philadelphia, Pennsylvania,* 1945.

Modern facial prosthesis is a product of the relatively recent development of materials to meet an increasing need. Although it is the plastic surgeon who is most frequently confronted with the necessity of utilizing a prosthesis either as a temporary or a permanent means of dealing with facial losses, most of the workers in this field have arisen within the dental profession as an inevitable extension of their familiarity with intra-oral prosthesis.

This book was written, according to its author, as a practical guide for those in the medical, dental and allied fields who may be called upon to do prosthetic facial reconstructions, and in the hope that it might serve as a text for students. About one half of it is devoted to the fundamental principles of prosthetic restorations and the technical handling of the materials available. The remainder presents more detailed and specific information on restoring the various features. The style of the book is simple and clear and the text is fully illustrated. Bulbulian is to be congratulated on his contribution to the subject.

E. M. H.

Ambulatory Proctology. By Alfred J. Cantor. 524 pp. $8.00. *Paul B. Hoeber, Incorporated, New York, New York,* 1946.

In this volume, the author makes a real contribution not only to proctology but to gastroenterology in emphasizing and formulating the techniques which can be carried out "without confining the patient to bed." His concept of the ambulatory proctologist is not necessarily one who treats all his patients "without the knife" or in his office by strictly "office methods." Rather, he is meeting in a practical way the problem of inadequate hospital facilities, the desire of people to be ambulatory and, if possible to keep on the job and also he is modifying, along with so many physicians, Hilton's old conception of the necessity of rest in treating most human ills. Hence, throughout the text one finds an emphasis upon anesthetic methods and especially upon the use of the oil-soluble anesthetics for their long lasting effect. To one who has never practised ambulatory methods to the extent advocated here, some of the procedures such as "extensive hemorrhoidectomies" seem rather ambitious. Apart from the pain, we all know that there are many untoward reactions and complications which can occur to plague the patient and the surgeon.

The text covers well, and in detail, the general field of diagnosis of diseases of the colon, rectum and anus and the treatment of those diseases which might be

considered to be ambulatory. Certainly, the author does not intend that the procedures should always be carried out in an ambulatory fashion on all patients, and so states. One might take exception to his critical views about the therapeutic use of alcohol injections in anal pruritus, a method which has been employed for years in the Johns Hopkins Clinic with success.

The volume bears evidence to the author's scientific approach and his originality; though in addition, the work contains abundant references, and ample credit is given to the opinions of others. His creative instinct and his mechanical ingenuity are beautifully illustrated by his telescopic sigmoidoscope, originally described in 1940 and his tattoo instrument for use in the therapy of anal pruritus. The volume is well set up and nicely illustrated with photographs and line drawings. In general, it is a well conceived and well prepared text, written with a broad gastroenterological viewpoint emphasizing the simplification (for the patient) of proctologic procedures. Any surgeon doing proctology will do well to familiarize himself with this book.

S. McL.

Physical Chemistry of Cells and Tissues. By RUDOLPH HÖBER. Illus. 676 pp. $9.00. *The Blakiston Company, Philadelphia, Pennsylvania,* 1945.

This book is not a revision of the author's well-known classic entitled "Physikalische Chemie der Zelle u. der Gewebe," but is a new book written in collaboration with other experts in the field. Of the thirty-eight chapters, Dr. Höber has, himself, contributed nineteen or exactly half of the total number.

The book is sub-divided into eight sections written by five different authors. The first section is entitled "Selected Principles of Physical Chemistry" and is by Dr. David I. Hitchcock of the Yale University. This section chooses examples of biologically important basic principles of physical chemistry. The section contains considerable mathematical detail. The mathematical treatment of the subject is too complicated for biologists who do not have a very extensive mathematical background. Clarification by examples and by making the subject matter more definitive would have helped the readability of the section. The reader gets the impression that this section is to some extent a condensation of Dr. Hitchcock's own textbook on the subject. The second section by Dr. J. B. Bateman of the Mayo Clinic is somewhat more readable, but also requires a comprehensive mathematical outlook for its complete digestion. This section is entitled "Large Molecules" and deals with electrical and chemical forces which determine the properties of such molecules and their inter-action. A discussion of x-ray methods and of surface chemistry is also included. This last cited chapter is of special significance.

With this purely technical background the next three sections written by Dr. Höber follow. These discuss the architecture of protoplasm, the surface of the protoplast, and the influence of certain extracellular factors on cellular activity. These are very well written and show the true genius of the editor of the volume.

The sixth section on "The Respiration of Cells and Tissues" is very good. The discussion of the Pasteur effect in Chapter 29 deserves especial commendation for

clarity. The seventh section on "Contractility" by Dr. Wallace O. Fenn is excellent but suffers from having been written in 1942. Since that time many aspects of contractility have been clarified by Engelhardt and others. The biochemical properties of muscle proteins such as myosin are a focal point of discussion concerning contractility since 1942.

The final section by Dr. Höber is entitled "Passive Penetration and Active Transfer in Animal and Plant Tissues" and it includes such subjects as intestinal absorption, the formation of urine, and the elaboration of digestive juices.

This book will prove very useful and all doctors who look to the future should at least glance within its pages. It suffers some from publication difficulties attendant on the war period during which it was prepared. Section 2 was received in 1943 and Section 7 in 1942, despite a publication date of 1945. The book is highly theoretical in many places and if any particular point is not clear to the reader, he has only one recourse; namely, to look up that subject in one or more of the many excellent references given. The book, however, in places makes little effort to clarify difficult points even for those well trained in the field. Examples are few, pictures are absent except for one or two diagrams of theoretical molecules. The book is in no sense a laboratory text and is essentially a theoretical presentation. On the other hand, the importance of the subject presented makes it one that should be of interest to all chemists, physicists and biologists. The editor definitely outlines his plan in the introduction and fulfills his objectives. Despite the theoretical aspect of the book, many of the chapters are of definite practical interest. Thus, Chapter 23 on "The Influence of Narcotics on Cell Activity" should be of especial interest to anesthetists both clinical and experimental. Anesthetics should also be interested in Section 6 entitled "Respiration of Cells and Tissues." The gastro-enterologist will be especially interested in the chapters on intestinal absorption and the formation of digestive juices.

This book shows once again that the dividing line between chemists and biologists is becoming more indistinct. In one century the cycle has been completed, and the day when men must again like Helmholtz be leaders in physical chemistry and in medicine at the same time is now at hand.

This is an important book. It cannot be read hurriedly, especially by one without a physico-chemical and mathematical background. All those who study it, however, both clinicians and biologists, will profit in proportion to the effort that they expend in reading it.

H. N. H.

A History of Medicine. By DOUGLAS GUTHRIE. Illus. 448 pp. $6.00. *J. B. Lippincott Company, Philadelphia, Pennsylvania,* 1946.

This book on the history of medicine by Dr. Douglas Guthrie of Edinburgh, has appeared at a most opportune time. During the war medical students were forced by the accelerated system to concentrate on studies that would be of value in casualty aid stations. The cultural influence and value of medical history were

side-stepped. With the appearance of Guthrie's book, all those who have neglected the study of medical history can make good that lack.

This book is not only authoritative, but it is extremely easy to read. The plates are excellent. It is just the right length to be complete and yet at the same time it does not frighten the prospective reader away by being encyclopedic. The footnotes are mainly bibliographical and at the end of each chapter is a summary of suggested books for further reading. A minor criticism might be made that these references are so brief that they do not include the name of the publisher of the respective books and this omission might handicap some who intend to look up the references for the suggested further reading. Individual leaders of medical history are talked about in a personal manner, but these biographical accounts are well fused into a unified whole. The last three chapters of the book are of special interest and cover the subjects of military and naval surgery and the conquest of tropical diseases, the rise of specialism and preventive medicine, and finally, an account of medical journalism, bibliography, and medical history. The appendix serves as a useful guide to all those who wish to pursue the subject further. The knowledge contained in this book is of such importance as to be of value to all medical men. It can be heartily recommended.

H. N. H.

Carbohydrate Metabolism. By SAMUEL SOSKIN and RACHMIEL LEVINE. 315 pp $6.00. *University of Chicago Press, Chicago, Illinois,* 1946.

In a rapidly advancing world of scientific knowledge, it is well, from time to time, to pause and take stock of the physiologic background of some of the clinical concepts on which we base treatment of disease. Such a review in the case of carbohydrate metabolism is the avowed purpose of the book by Drs. Soskin and Levine. Their purpose is admirably achieved. The newer studies of cellular physiology based on enzyme chemistry are set forth in logical order and delightfully readable style. Particularly pleasing is the excellence of the bibliographic references. Controversial matter is treated with fairness, especially in view of the long-standing bias of the authors in favor of the "overproduction theory"—that fat is converted to carbohydrate in excessive quantities in diabetes. Though the actual care of the patient with diabetes mellitus is not notably altered by these newer concepts, the doctor should be fully aware of them in order to best outline his therapeutic program. The book can be recommended wholeheartedly to practitioners and students.

J. E. H.

"The Cortex of Galago; Its Relation to the Pattern of the Primate Cortex." By GERHARDT VON BONIN. Illus. 56 pp. *University of Illinois Press, Chicago, Illinois,* 1945.

This handsomely printed monograph describes the brain of a small African lemur in considerable detail. It is a purely morphological description, except for a few

mathematical and philosophical overtones. There is no particular attempt at physiological correlation. Only a single specimen was studied. It may prove of some interest to comparative anatomists, although there are already similar studies available. The clinician will find it hard to see how any possible usefulness of such a publication could ever justify the time and expense involved in its production.

G. G. M.

Wm. Beaumont's Formative Years: Two Early Notebooks 1811–1821. Illus. 87 pp. $6.00. *Henry Schuman, New York, New York,* 1946.

This historical volume is beautifully bound and printed and contains a number of plates depicting scenes from Beaumont's time and locale. The publishers and the editor are to be congratulated on producing this book.

Essentially the volume represents two diaries and notebooks of William Beaumont. These cover the period from Dr. Beaumont's medical apprenticeship in St. Albans, Vermont, through and including his period of military service during the war of 1812, and conclude with his first trip to Mackinac Island. While portions of the diary are incomplete and illegible, the literal publication of such a work gives a very direct insight into Beaumont's character. Such items as medical prescriptions, quotations from classic authors that appealed to Beaumont and notes on conditions of the day are all included. Several pages are occupied with Beaumont's attempt to carry out Benjamin Franklin's project for obtaining moral perfection.

The editor has included one hundred seventy-two annotations which indicate very careful research into the period covered by the notebooks. The only possible criticism that this reviewer can find with the book is that the annotations are grouped at the end of the notebooks rather than being on each page; thus the careful reader must turn to the back of the book one hundred seventy-two times. On the other hand, the fact that the pages themselves contain nothing but Beaumont's writing gives a more natural atmosphere to the text.

William Beaumont still stands as one of America's greatest medical men. All those interested in American medical history will enjoy seeing this book.

H. N. H.

Through the Stratosphere: The Human Factor in Aviation. By MAXINE DAVIS. 253 pp. $2.75. *The Macmillian Company, New York, New York,* 1946.

This book is an enthusiastic account in popular style of the varied activities of the U. S. Army Air Forces Medical Service during World War II. The writer, by virtue of access to official reports and publications and considerable personal observation in the field has been able to present an accurate and dramatic picture of many problems encountered by flight surgeons and of the successful solutions developed by the vast organization of the USAAF. The story unfolded here is at once a tribute to the ingenuity of the chemists, engineers, physicians, physicists, physiologists and psychologists and at the same time a subtle warning that their

major triumphs, which were in the domain of applied or industrial medicine, left unanswered a host of fundamental questions. The urgent need for immediate answers to critical problems has well-nigh exhausted the reservoir of basic information required to protect man against the tremendous physical and emotional stresses which modern military aviation promises to create in the very near future.

It would seem worthwhile to suggest here that the generally held concept of aviation medicine as "... a new- and now essential-specialty: care of fliers" is fostered in the face of the obvious fact that few fields of medicine employ as many diverse disciplines and technics: aviation medicine is rather the application of many specialties, medical, physical, chemical, engineering, to an unusual and difficult problem in industrial hygiene.

J. L. L. Jr.

BOOKS RECEIVED FOR REVIEW

Developmental Anatomy. A Textbook and Laboratory Manual of Embryology. By LESLIE BRAINERD AREY. 5th Ed. Illus. 616 pp. $7.00. W. B. Saunders, Philadelphia, Pennsylvania, 1946.

Early Ambulation and Related Procedures in Surgical Management. By DANIEL J. LEITHAUSER. 232 pp. $4.50. Charles C Thomas, Springfield, Illinois, 1946.

Jewish Luminaries in Medical History. By HARRY FRIEDENWALD. 199 pp. $3.00. The Johns Hopkins Press, Maryland, 1946.

Manual of Applied Nutrition. The Johns Hopkins Hospital. 103 pp. $1.50. Baltimore, Maryland, 1946.

Medical Biochemistry. By MARK R. EVERETT. 2nd Ed. $7.00. 765 pp. Paul B. Hoeber, Incorporated, New York, New York, 1946.

Penicillin: Its Practical Application. Illus. 380 pp. $7.00. Blakiston Company, Philadelphia, Pennsylvania, 1946.

Practical Malariology. By PAUL F. RUSSELL, Luther S. West and Reginald D. Manwell. Illus. 684 pp. $8.00. W. B. Saunders, Philadelphia, Pennsylvania, 1946.

Primer for Diabetic Patients. By RUSELL M. WILDER. 8th Ed. Illus. 192 pp. $1.75. W. B. Saunders Company, Philadelphia, Pennsylvania, 1946.

Professional Adjustments. By SISTER MARY ISIDORE LENNON. Illus. 299 pp. $3.00. The C. V. Mosby Company, St. Louis, Missouri, 1946.

Psychotherapy in General Medicine: Report of an Experimental Postgraduate Course. By GEDDES SMITH. 38 pp. $.25. The Commonwealth Fund, New York, New York, 1946.

INDEX TO VOLUME LXXIX

Pagination according to months:
July, 1946, 1–96
August, 1946, 97–205
September, 1946, 207–259
October, 1946, 261–331
November, 1946, 333–363
December, 1946, 365–484

Abdomen, Wounds of 283
524 Abdominal Wounds on the Western Front. Jones, Howard W. and Falor, William H. 283
Anoxemia, Effect on Man 70
Anoxemia, Effect on Rats 59

Beadenkopf, W. G. (See Kibbe, F. W.) 365
Billings, F. Tremaine (See Winkenwerder, Walter L.) 406
Birmingham, Marion (See Newman, E. V.) 229
Blackman, Sam (See Lyford, John III) 349
Blain, Alexander, III: (See Calihan, Robert J.) 21
Blain, Alexander, III: The Effect of Penicillin in Experimental Intestinal Obstruction. Studies on Strangulated Low Ileal Obstructions 1
Bliss, Eleanor A. (See Wilson, Catherine E.) 451
Bone, Granuloma Inguinale of 349
Book Reviews 253, 320, 470
Brown, N. Worth: Letter to the Editor 202
Byrne, Ann F. (See Wilson, Catherine E.) 451

Calihan, Robert J.: Intestinal Obstruction: A Study of Two Hundred and Four Acute Cases with Reference to the Possible Efficacy of Anti-Bacterial Therapy ... 21
Carroll, Douglas G. (See Winkenwerder, Walter L.) 406
Clinical Significance of Certain Changes in the Limb Lead Electrocardiogram in Arterial Hypertension, A Clinico-Pathologic Study. Filley, Giles F. 261
Clinton, Marshall, Jr. (See Thorn, George W.) 59
Clinton, Marshall, Jr.: Studies on Altitude Tolerance. II. Studies on Normal Human Subjects—Effect of Repeated Short Exposures to Reduced Atmospheric Pressure 70
Cochlea, Lesions of, Effect on Auditory Cortical Responses 309
Cole, Rufus: Perfectionism in Medicine 190
Consciousness, Localization in Brain 34
Control of Experimental Pneumonia with Penicillin. Catherine E. Wilson, Carolyn W. Hammond, Ann F. Byrne, and Eleanor A. Bliss 451
Corpus Striatum 34
Cortex, Auditory, Effects of Cochlear Lesions on Response 309

Dandy, Walter E.: Location of the Conscious Center in the Brain: the Corpus Striatum 34

Davenport, Virginia D. (See Clinton, Marshall, Jr.) 70
Differences in the Action of Crystalline and Non-crystalline Preparations of Estriol. Schiller, Joseph 243

Eagle, Harry: Effect of the Method of Administration on the Therapeutic Efficacy of Sodium Penicillin in Experimental Syphilis 168
Edmonds, H. W. (See Thorn, George W.) 59
Effects of Cochlear Lesions on Click Responses in the Auditory Cortex of the Cat. Walzl, Edward M. and Woolsey, Clinton N. 309
Effect of Penicillin in Experimental Intestinal Obstruction. Studies on Strangulated Low Ileal Obstruction. Blain, Alexander, III and Kennedy, John D. 1
Effect of the Method of Administration on the Therapeutic Efficacy of Sodium Penicillin in Experimental Syphilis. Eagle, Harry, Magnuson, Harold and Fleischman, Ralph 168
Electrocardiographic Changes in Hypertension 261
Estriol, Differences in Action of Preparations 243

Falor, William H. (See Jones, Howard W.) 283
Farber, S. (See Thorn, George W.) 59
Filley, Giles, F. Clinical Significances of Certain Changes in the Limb Lead Electrocardiogram in Arterial Hypertension, A Clinico-Pathologic Study 261
Fleischman, Ralph (See Eagle, Harry) 168
Fuadin, Treatment of Schistosomiasis Japonica 406

Gilman, A. (See Newman, E. V.) 229
Glomerulonephritis, Simulation by Sarcoidosis 333
Granuloma, Inguinale, Disseminated 349

Hammond, Carolyn W. (See Wilson, Catherine E.) 451
Harper, W. Henry: Necrosis and Ulceration of the Intestinal Wall in Simple Intestinal Obstruction. An Experimental Study 207
Harrison, Thurston (See Winkenwerder, Walter L.) 406
Hartman, Thomas L.: Sulfonamide Sensitivity Determinations of Hemolytic Streptococci Isolated from Patients before and after Treatment with Sulfadiazine 342
Heart, Standstill After Carotid Sinus Pressure 202
Hunninen, Arne V. (See Winkenwerder, Walter L.) 406
Hypertension, Significance of Electrocardiographic Changes 261

Intestinal Obstruction: A Study of Two Hundred and Four Acute Cases with Reference to the Possible Efficacy of Anti-Bacterial Therapy. Calihan, Robert J., Kennedy, John D. and Blain, Alexander, III 21
Intestinal Obstruction, Effect of Penicillin 1
Intestinal Obstruction, Necrosis and Ulceration of Intestinal Wall in 207
Intestinal Obstruction, Study of Acute Cases 21

Johns Hopkins Medical Society, Proceedings of Meetings 90, 247, 358, 467
Johnson, Robert W., Jr. (See Lyford, John, III) 349
Joint, Granuloma Inguinale of 349
Jones, Howard W.: 524 Abdominal Wounds on the Western Front 283

Kennedy, John D. (See Blain, Alexander III) 1
Kennedy, John D.: (See Calihan, Robert J.) 21
Kibbe, F. W.: A Report on an Epidemic of Mild Lymphocytic Meningitis in Burma 365
Kidney, Clearance of Thiosulfate 229
Klinefelter, Harry F., Jr.: Sarcoidosis Simulating Glomerulonephritis 333

Lemmer, Richard A. (See Harper, W. Henry) 207
Letter to the Editors. Brown, N. Worth 202
Location of the Conscious Center in the Brain—the Corpus Striatum. Dandy, Walter E. 34
Lyford, John, III: Pathologic Findings in a Fatal Case of Disseminated Granuloma Inguinale with Miliary Bone and Joint Involvement 349

Maier, John (See Winkenwerder, Walter L.) 406
Magnuson, Harold J. (See Eagle, Harry) 168
Meningitis, Lymphocytic, Epidemic 365

Necrosis and Ulceration of the Intestinal Wall in Simple Intestinal Obstruction. An Experimental Study. Harper, W. Henry and Lemmer, Richard A. 207
Newman, E. V.: Renal Clearance of Thiosulfate in Man 229

Pathologic Findings in a Fatal Case of Disseminated Granuloma Inguinale with Miliary Bone and Joint Involvement. Lyford, John III, Johnson, Robert W., Jr., Blackman, Sam and Scott, Roger B. 349
Penicillin in Experimental Syphilis 168
Penicillin in Experimental Intestinal Obstruction 1
Perfectionism in Medicine. Cole, Rufus 190
Philips, F. S. (See Newman, E. V.) 229
Pneumonia, Atypical, Transmission to Man 59
Problem of Identification of Oral Spirochetes and Description of a Precipitin Test for Their Serological Differentiation. Robinson, Lucille B. and Wichelhausen, Ruth 436

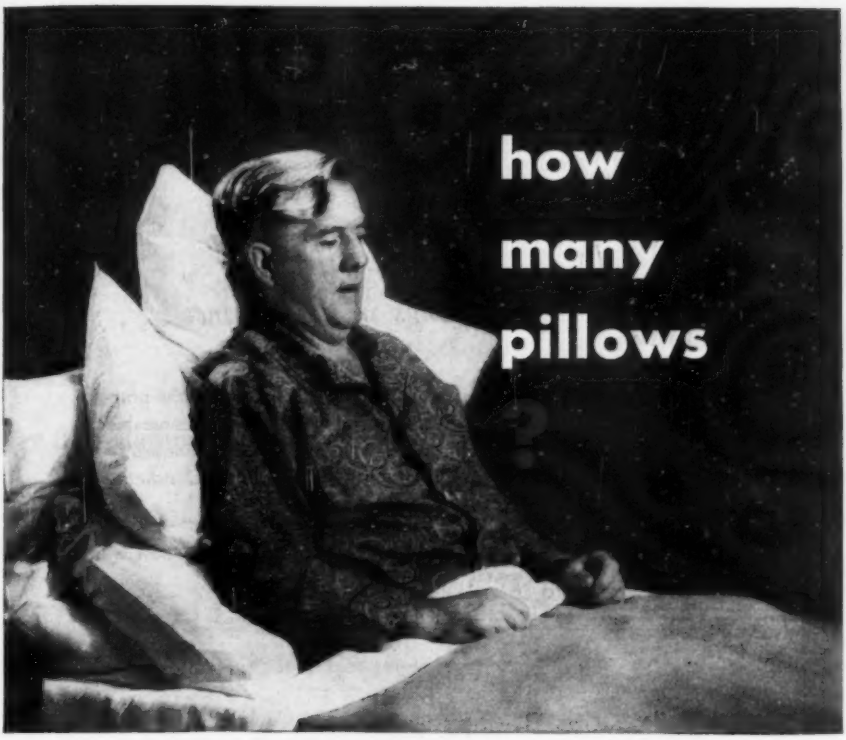
Renal Clearance of Thiosulfate in Man. Newman, E. V., Gilman, A. and Philips, F. S. With technical assistance of Robinson, Margot and Birmingham, Marion. 229
Report on an Epidemic of Mild Lymphocytic Meningitis in Burma. Kibbe, F. W. and Beadenkopf, W. G. 365
Robinson, Lucille B.: The Problem of Identification of Oral Spirochetes and Description of a Precipitin Test for Their Serological Differentiation 436
Robinson, Margot. (See Newman, E. V.) 229

Salley, S. Marion. (See Klinefelter, Harry, F., Jr.) 333
Sarcoidosis Simulating Glomerulonephritis. Klinefelter, Harry F., Jr. and Salley, S. Marion 333
Schiller, Joseph: Differences in the Action of Crystalline and Non-crystalline Preparations of Estriol 243
Schistosomiasis Japonica, Treated with Fuadin 406
Scott, Roger B. (See Lyford, John III) 349

Spirochetes, Oral, Identification by Precipitin Test..... 436
Streptococci, Hemolytic, Sensitivity to Sulfonamide..... 342
Studies on Altitude Tolerance. I. Studies on Normal Rats—Effect of Repeated Short Exposures to Reduced Atmospheric Pressure. Thorn, G. W., Clinton, M. Jr., Farber, S. and Edmonds, H. W..... 59
Studies on Altitude Tolerance. II. Studies on Normal Human Subjects—Effect of Repeated Short Exposures to Reduced Atmospheric Pressure. Clinton, Marshall, Jr., Thorn, George W. and Davenport, Virginia D..... 70
Studies on Schistosomiasis Japonica: 2. Analysis of 364 Cases of Acute Schistosomiasis with Report of Results of Treatment with Fuadin in 184 Cases. Winkenwerder, Walter L., Hunninen, Arne V., Harrison, Thurston, Billings, F. Tremaine, Carroll, Douglas G. and Maier, John..... 406
Sulfonamide Sensitivity Determinations of Hemolytic Streptococci Isolated from Patients before and after Treatment with Sulfadiazine. Hartman, Thomas L.. 342
Syphilis, Effect of Penicillin on Experimental Disease..... 168

Thiosulfate, Renal Clearance in Man..... 229
Thorn, George W. (See Clinton, Marshall, Jr.)..... 70
Thorn, George W.: Studies on Altitude Tolerance. I. Studies on Normal Rats—Effects of Repeated Short Exposures to Reduced Atmospheric Pressure..... 59
Transmission of Primary Atypical Pneumonia to Human Volunteers. I. Experimental Methods. Commission on Acute Respiratory Diseases..... 97
Ibid. II. Results of Inoculation..... 109
Ibid. III. Clinical Features..... 125
Ibid. IV. Laboratory Studies..... 153

Walzl, Edward M.: Effects of Cochlear Lesions on Click Responses in the Auditory Cortex of the Cat..... 309
Wichelhausen, Ruth. (See Robinson, Lucille B.)..... 436
Wilson, Catherine E.: The Control of Experimental Pneumonia with Penicillin ... 451
Winkenwerder, Walter L.: Studies on Schistosomiasis Japonica: 2. Analysis of 364 Cases of Acute Schistosomiasis with Report of Results of Treatment with Fuadin in 184 Cases..... 406
Woolsey, Clinton N.: (See Walzl, Edward M.)..... 309



The degree of orthopnea is a reliable aid
in the evaluation of cardiorespiratory impairment.
In chronic congestive heart failure,
as well as in emergencies, Searle Aminophyllin decreases
the myocardial burden and thereby improves the
efficiency of the contractions.
Administered orally, parenterally or rectally.

ACCEPTED AMERICAN MEDICAL ASSN.

AMINOPHYLLIN

contains at least 98% of anhydrous theophylline. G. D. Searle & Co., Chicago 80, Illinois

SEARLE / RESEARCH IN THE SERVICE OF MEDICINE

(In writing advertisers, please mention the Bulletin.)

When a Life
Hangs in the Balance . . .

. . . and progressive wasting increases the gravity of the prognosis, depletion of body proteins can be prevented. Parenamine —parenteral source of the indispensable and other amino acids— provides the elements of protein nutrition . . . sustains the regenerative processes essential to recovery.

Parenamine
Amino Acids Stearns
PARENTERAL

For Protein Deficiency

PARENAMINE is a sterile 15 per cent solution of amino acids containing all known to be essential for humans, derived by acid hydrolysis from casein and fortified with pure *dl*-tryptophane.

INDICATED in conditions of restricted intake, faulty absorption, increased need or excessive loss of proteins such as in pre- and postoperative management, extensive burns, delayed healing, gastro-intestinal disorders, et cetera.

ADMINISTRATION may be intravenous, intrasternal or subcutaneous.

SUPPLIED as 15 per cent sterile solution in 100 cc. rubber-capped bottles.

Reprints and complete clinical data will gladly be sent on request.

Frederick Stearns & Company
Division
DETROIT 31, MICHIGAN

NEW YORK · KANSAS CITY · SAN FRANCISCO · WINDSOR, ONTARIO · SYDNEY, AUSTRALIA · AUCKLAND, NEW ZEALAND

Trade-Mark Reg. U. S. Pat. Off.

RADIUM

For the Treatment of Deafness Caused by Nasopharyngeal Lymphoid Tissue

The Radium Chemical Company, Inc., offers a Radium Nasopharyngeal Applicator, constructed in accordance with specifications of physicians specializing in this particular field for many years.

The technic of application has been proved, and this applicator is now used by more than 500 clinics and specialists throughout the United States.

Its advantages are:

1: *Only 50 milligrams of radium required for treatment.*

2: *The radium capsule has an external diameter of 2.3 millimeters, and the handle is semi-flexible. This permits easy insertion into the nasopharyngeal passages of small children.*

3: *The thin wall of the radium capsule allows the emission of a sufficient volume of beta rays to reduce materially the treatment time.*

Special lead cases are available for storage and transportation from office to hospital.

Write for complete details. No obligation.

RADIUM CHEMICAL CO., INC.

570 Lexington Ave., New York 22, N. Y.

FOUR TREATISES OF THEOPHRASTUS VON HOHENHEIM CALLED PARACELSUS

The volume was issued by the Institute of the History of Medicine in commemoration of the 400th anniversary of the death of Paracelsus.

CONTENTS

Preface: HENRY E. SIGERIST.

I. Seven Defensiones, the Reply to Certain Calumniations of His Enemies.
Translated from the German, with an Introduction by C. LILIAN TEMKIN.

II. On the Miner's Sickness and Other Miners' Diseases.
Translated from the German, with an Introduction by GEORGE ROSEN.

III. The Diseases That Deprive Man of His Reason, Such as St. Vitus' Dance, Falling Sickness, Melancholy, and Insanity, and Their Correct Treatment.
Translated from the German, with an Introduction by GREGORY ZILBOORG.

IV. A Book on Nymphs, Sylphs, Pygmies, and Salamanders, and on the Other Spirits.
Translated from the German, with an Introduction by HENRY E. SIGERIST.

Literature.

xiv + 256 pages, frontispiece, octavo, cloth, $3.00.

THE JOHNS HOPKINS PRESS · BALTIMORE 18

PUBLICATIONS OF THE INSTITUTE OF THE HISTORY OF MEDICINE
THE JOHNS HOPKINS UNIVERSITY

FIRST SERIES: MONOGRAPHS
Volumes II and III

THE JEWS AND MEDICINE
ESSAYS

BY

HARRY FRIEDENWALD

The present volumes that contain the collected essays of Dr. Harry Friedenwald represent not only a contribution to the history of medicine among the Jews and to the history of medicine practised by Jewish physicians; they are a great contribution to the history of medicine and to the history of learning at large.

Dr. Harry Friedenwald is the outstanding expert in the field and possesses a great collection of manuscripts, incunabula, books and documents pertaining to the subject

1944, xxiv + 817 pages, illustrated, $7.50.

THE JOHNS HOPKINS PRESS · BALTIMORE 18, MARYLAND

(In writing advertisers, please mention the Bulletin.)

BASAL ANESTHESIA

CUTS ETHER REQUIREMENT 1/2

Basal anesthesia by means of rectal instillation of Avertin with amylene hydrate decreases by one-half the amount of inhalation anesthetic subsequently needed.

This is of particular value in thyroid, brain and facial surgery where the ether cone or gas mask represents a great inconvenience.

It is also of importance in the presence of pulmonary complications. Avertin with amylene hydrate can not cause pulmonary irritation since it is excreted quantitatively by the kidneys.

Write for detailed information.

AVERTIN

Trademark Reg. U.S. Pat. Off. & Canada

BRAND OF TRIBROMOETHANOL

with Amylene Hydrate

Winthrop

CHEMICAL COMPANY, INC. Pharmaceuticals of merit for the physician · New York 13, N. Y. · Windsor, Ont.

IT DOES HAPPEN HERE

Severe rickets still occurs—even in sunny climates

Vitamin D has become such an accepted practice in infant feeding that it is easy to think that rickets has been eradicated. However, even deforming rickets is still seen, as witness the above three contemporary cases from three different sections of the United States, two of them having well above the average annual sunshine hours for the country. In no case had any antiricketic been given during the first two years of life. *It is apparent that sunlight did not prevent rickets.* In other cases of rickets, cod liver oil was given inadequately (drop dosage) and even this was continued only during the winter months.

To combat rickets simply, inexpensively, effectively—

OLEUM PERCOMORPHUM

This highly potent source of natural vitamins A and D, if administered regularly from the first weeks of life, will not only prevent such visible stigmata of rickets as pictured above, but also many other less apparent skeletal defects that might interfere with good health. What parent would not gladly pay for this protection! And yet the average prophylactic dose of Oleum Percomorphum costs less than one cent a day. Moreover, since the dosage of this product is measured in drops, it is easy to administer Oleum Percomorphum and babies take it willingly. Thus there is assurance that vitamin D will be administered *regularly.*

EXIGENCY OF WAR

Oleum Percomorphum 50% is now known as Oleum Percomorphum With Other Fish Liver Oils and Viosterol. A source of vitamins A and D in which not more than 50% of the vitamin D is derived from Viosterol. The potency remains the same; namely, 60,000 vitamin A units and 8,500 vitamin D units per gram.

MEAD JOHNSON & COMPANY, Evansville 21, Indiana, U.S.A.